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JOURNAL OF ANIMAL SCIENCE

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Published Quarterly

by the

American Society of Animal Production

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Volume 12 Number 3

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THE RELATION BETWEEN SALE PRICE AND MERIT IN COLUMBIA, TARGHEE AND RAMBOUILLET RAMS

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THE determination of relative economic value of traits emphasized in breeding and selection is a fundamental problem of animal breeders. This problem can be attacked by obtaining the relationship of sale price to merit of animals sold, since the buyers' willingness to pay more for animals of greater apparent merit in specific traits is one measure of the worth of these traits. Of course, the purchase of stud rams is an intermediate step in the production of market lambs and wool, but the real objectives are fundamentally the same for sheep breeders as for commercial producers of lambs and wool.

Improvement of sheep through selection is largely dependent on the selection of rams as shown by Terrill (1951) and Dunlop (1951). Thus, the buyer of rams is mainly dependent on the ram breeder for any improvement from selection. The buyer also is limited by his ability to purchase the better rams of those available. The furnishing of comparable production records on sale rams is one means by which the ram breeder can assist the buyer in improving his flock through the selection of rams.

Since 1946 the U. S. Sheep Experiment Station has made some production records on stud rams routinely available to prospective buyers. In addition all records at the Station are available to the public, and many buyers ask for additional information on rams they are interested in buying. This practice appears to be increasing in recent years.

The objectives of this study are to determine the relation of sale price to records of merit as an estimate of value attached to improvement in these traits by the buyer of stud rams, and to obtain indications of possible ways to encourage the use of production records in the improvement of sheep by selection.

Material and Methods

Single stud rams sold at public auction at this Station from 1946 through 1951 were included in this study. These auction sales were

[1] U. S. Sheep Experiment Station and Western Sheep Breeding Laboratory, Dubois, Idaho, in cooperation with the Idaho Agricultural Experiment Station.

held on the last Thursday in September each year and were well attended by sheep breeders and producers from many states. Records from 202 Columbia, 104 Targhee and 59 Rambouillet rams were studied.

Records presented in the sales catalogue included eartag number, registration number if available for registry, date of birth, grease and clean fleece weight in pounds and staple length in inches. The latter three records were lifetime averages adjusted for important measurable environmental effects such as type of birth and age of dam, plus inbreeding, age, year and number of records by the method described by Terrill (1948). In 1950 and 1951 these records of rams 2 years old and older were also adjusted for previous selection practiced. The purpose of the adjustments was to make comparisons between individual rams as accurate as possible. Presence of scurs on Columbia rams was also indicated in the sale catalogue.

Other records studied that were available to prospective buyers on request were: index; fleece grade expressed in blood grades at shearing; fleece grade in spinning counts based on fiber diameter of a sample cross section; body weight after shearing adjusted as above; body weight in August or September; scores for type, condition and face covering taken near time of previous shearing; age of yearling rams in days to shearing (days from shearing to sale were constant for all rams each year); age of rams older than yearlings in years; presence of scurs; type of birth; and age of dam. The scoring system was the same for the three traits evaluated by scoring; rams with highest merit were given a score of 1 and those with lowest merit a score of 5. Fifteen possible scoring units were obtained by assigning plus or minus values where merit appeared to be slightly above or below a whole unit. Independent scores were taken by three men and average values were used.

The index used for Rambouillet and Targhee rams, based on adjusted lifetime averages for each trait, equals body weight in pounds minus 3 times grease fleece weight in pounds plus 11 times clean fleece weight in pounds plus staple length in centimeters plus 7 times type score plus 4 times condition score minus 7 times face covering score minus 3 times neck folds score. Negative factors in the index do not necessarily indicate negative emphasis because of relationships among traits.

The index used for Columbia rams based on adjusted lifetime averages for each trait equals grease fleece weight in pounds plus 18 times clean fleece weight in pounds plus 2 times staple length in

centimeters minus 2.5 times fineness code plus body weight in pounds plus 6 times type score plus 3 times condition score. The fineness codes range from 1 to 12 for fleeces grading 70's to 36's, respectively.

Correlation coefficients were calculated separately for yearling and older rams on a within-year basis. In Rambouillets, correlations were calculated on a within-group basis with registered and unregistered and polled and horned rams being grouped separately within years.

Results and Discussion

Means by year and age group and the within-year standard deviations for the various records studied are presented in tables 1, 2 and 3. The means of adjusted records are, of course, fairly constant from year to year. Average prices vary considerably among years and in general have increased during the last 3 years. In most of the groups, highest prices were obtained in 1951 while second highest prices were obtained in 1950.

Correlation coefficients showing relationship between sale price and the various records studied are presented in table 4.

Rams were generally sold in order, according to their rank in general appearance; there were some exceptions, particularly in the case of small groups of mature rams where the rams were sometimes sold in a random order. The relation of price to sale order was significant only for Columbia yearling rams where the rams selling earlier tended to bring higher prices than those sold later.

Buyers tended to pay more for rams with heavy grease fleece weights as shown by positive correlations between price and grease fleece weight in five of the six groups, three of which were significant. A similar tendency was shown for clean fleece weight where positive correlations were obtained in four of the six groups, two of which were significant. None of the correlation coefficients between price and staple length were significant, although all but one indicated that slightly higher prices were paid for rams with longer staple.

Correlations of fineness, based on cross section fiber diameter, with sale price were all low and non-significant. Negative correlations for Columbias and Targhees indicate an advantage for rams with slightly finer fleeces in these breeds, while positive correlations for Rambouillets indicate an advantage for rams with slightly coarser fleeces. An examination of the distribution of prices according to blood grades of fleeces of single sale rams did not reveal any consistent relationship of price with blood grade.

TABLE 1. AVERAGE RECORDS FROM COLUMBIA RAMS

Year of Sale	No. of Head	Price	Adj. Grease Fleece Weight	Adj. Clean Fleece Weight	Adj. Staple Length	Adj. Body Weight	Fall Body Weight	Type	Condition	Face Covering	Index
		($)	(lb.)	(lb.)	(in.)	(lb.)	(lb.)	(score)	(score)	(score)	
					Rams of yearling age						
1946	23	167.83	17.8	10.4	4.65	193.7	160.9	1.75	1.77	2.73	420.6
1947	26	227.50	18.2	10.4	4.71	193.2	160.0	1.68	1.70	2.50	418.3
1948	27	132.59	17.9	10.3	4.62	196.0	160.1	1.77	1.77	2.63	414.1
1949	20	114.50	17.3	9.9	4.64	193.5	145.6	2.08	2.02	2.72	406.6
1950	21	244.05	17.4	10.2	4.56	186.4	160.2	1.98	1.92	2.79	405.2
1951	25	493.40	17.5	10.3	4.50	187.2	145.2	1.91	1.93	2.71	406.9
Std. dev. within yrs.		141.85	1.1	0.6	0.37	8.4	12.4	0.33	0.27	0.43	16.2
					Rams 2 years old and older						
1946	19	134.21	18.1	10.4	4.49	193.3	197.1	1.58	1.53	2.42	421.3
1947	11	165.45	17.8	10.4	4.53	191.2	192.0	1.73	1.67	2.15	412.7
1948	8	146.25	18.1	10.3	4.74	195.9	195.2	1.50	1.46	2.25	415.0
1949	10	94.00	17.9	10.4	4.76	192.6	170.9	2.17	2.23	2.83	414.1
1950	8	226.88	18.0	10.4	4.56	192.2	210.8	1.62	1.67	2.58	415.9
1951	4	585.00	18.3	10.6	4.48	190.5	210.0	1.33	1.25	2.92	417.2
Std. dev. within yrs.		152.29	1.2	0.8	0.33	7.2	18.4	0.42	0.41	0.48	17.4

TABLE 2. AVERAGE RECORDS FROM TARGHEE RAMS

Year of Sale	No. of Head	Price	Adj. Grease Fleece Weight	Adj. Clean Fleece Weight	Adj. Staple Length	Adj. Body Weight	Fall Body Weight	Type	Condition	Face Covering	Index
		($)	(lb.)	(lb.)	(in.)	(lb.)	(lb.)	(score)	(score)	(score)	
					Rams of yearling age						
1946	11	34.55	17.2	8.4	3.69	188.7	156.3	1.61	1.64	4.18	223.3
1947	9	183.33	17.7	8.8	3.88	188.2	158.9	1.74	1.70	3.33	228.4
1948	6	163.33	17.7	8.8	3.93	194.3	156.7	1.94	1.94	3.72	232.8
1949	15	147.00	17.1	8.7	4.09	188.8	155.9	2.13	2.02	3.47	230.5
1950	14	285.36	17.4	9.4	4.11	187.6	171.1	2.14	2.17	3.50	237.4
1951	22	396.82	17.1	9.3	4.08	183.5	162.6	2.01	1.91	3.39	234.9
Std. dev. within yrs.		106.47	1.3	0.6	0.29	7.4	14.1	0.39	0.33	0.58	9.6
					Rams 2 years old and older						
1946	10	38.00	16.3	8.1	3.57	190.3	194.6	1.43	1.37	3.23	229.1
1947	6	130.83	18.0	9.1	4.00	185.0	199.2	1.28	1.33	3.06	228.5
1948	5	186.00	18.0	9.0	3.92	196.0	194.8	1.40	1.26	3.14	235.2
1949	4	121.25	17.4	8.9	4.25	192.5	172.8	2.25	2.08	3.16	236.2
1950	4	168.75	16.2	8.5	4.12	182.2	193.0	1.66	1.66	3.16	228.0
1951	5	500.00	16.8	9.3	4.20	183.6	193.4	1.60	1.47	2.67	238.8
Std. dev. within yrs.		117.86	1.9	0.5	0.33	8.0	17.5	0.36	0.33	0.61	11.5

TABLE 3. AVERAGE RECORDS FROM RAMBOUILLET RAMS

Year of Sale	Group	No. of Head	Price	Adj. Grease Fleece Weight	Adj. Clean Fleece Weight	Adj. Staple Length	Adj. Body Weight	Fall Body Weight	Type	Condition	Face Covering	Index
			($)	(lb.)	(lb.)	(in.)	(lb.)	(lb.)	(score)	(score)	(score)	
						Rams of yearling age						
1948	Unregistered, horned	3	43.33	16.9	8.8	3.50	185.3	164.7	1.67	1.78	4.33	222.7
1949	Unregistered, polled	2	105.00	17.2	9.4	3.55	205.0	176.5	1.67	1.67	4.34	247.0
1950	Registered, horned	6	115.83	16.6	8.8	3.43	182.8	143.3	2.50	2.22	4.61	218.2
1950	Unregistered, horned	4	161.25	15.8	8.6	3.48	182.8	169.5	2.33	2.16	4.42	221.8
1950	Unregistered, polled	4	105.00	16.9	8.8	3.35	177.8	146.5	2.42	2.16	4.58	212.2
1951	Registered, horned	2	102.50	16.3	8.6	3.50	173.5	136.0	2.16	2.16	3.84	209.5
1951	Unregistered, horned	8	85.62	16.9	8.7	3.45	187.2	167.6	1.83	1.88	3.62	225.4
1951	Registered, polled	2	280.00	17.2	9.0	3.35	182.5	165.5	2.00	2.16	5.00	217.5
1951	Unregistered, polled	4	172.50	17.2	9.0	3.50	180.2	160.2	1.83	1.92	3.66	222.5
Standard deviation within groups			35.39	1.3	0.5	0.18	6.9	9.2	0.25	0.18	0.59	7.5
						Rams 2 years old and older						
1948	Unregistered, polled	3	105.00	17.5	9.1	3.53	172.0	165.0	1.89	1.44	4.22	213.3
1949	Registered, horned	8	40.94	17.9	9.0	3.49	186.4	177.4	2.08	2.12	4.62	220.1
1950	Unregistered, polled	5	161.00	16.6	9.3	3.66	176.2	185.8	1.67	1.67	4.33	219.8
1950	Unregistered, horned	2	150.00	16.2	9.8	4.20	181.5	192.5	1.50	1.50	4.00	234.5
1951	Unregistered, horned	3	91.67	16.7	9.0	3.73	177.3	186.3	1.89	2.00	3.11	221.3
1951	Unregistered, polled	2	235.00	17.0	9.0	3.50	189.5	197.5	1.50	1.50	4.34	228.0
Standard deviation within groups			44.14	1.3	0.8	0.25	6.6	13.1	0.44	0.45	0.56	8.4

TABLE 4. CORRELATION OF PRICES WITH VARIOUS RECORDS OF SINGLE SALE RAMS

Age Group	Breed	d/f	Correlation Coefficient of Price with: Order of Sale	Adj. Grease Fleece Weight	Adj. Clean Fleece Weight	Adj. Staple Length	Fineness Code	Adj. Body Weight	Fall Body Weight	Type	Condition	Face Covering	Index
Yearling	Columbia	136	—.35**	0.22**	0.19*	0.10	—.03	0.36**	0.49**	—.33**	—.32**	0.03	0.31**
Yearling	Targhee	70	—.09	0.15	0.21	—.12	—.08	0.24*	0.30*	—.07	—.07	—.01	0.23*
Yearling	Rambouillet	25	0.14	0.41*	0.26	0.18	0.21	0.73**	0.49*	—.49**	—.37	0.13	0.45*
Mature	Columbia	53	—.09	—.16	—.18	0.05	—.14	0.14	0.10	—.06	—.12	—.02	—.08
Mature	Targhee	27	0.16	0.06	—.12	0.02	—.03	—.04	0.01	—.25	—.05	0.14	—.18
Mature	Rambouillet	16	—.29	0.75**	0.79**	0.36	0.15	0.12	0.00	—.11	—.11	0.21	0.59*

* Significant at the .05 level.
** Significant at the .01 level.

Significant correlations between body weight and sale price were found in each of the yearling groups but not in the mature groups. Apparently less attention was paid to body weight in mature rams than in yearling rams. Correlations between price and body weight were as high as or higher than for any other trait in the three groups of yearling rams.

The correlations of type and condition scores with price were negative in every group, indicating that higher prices were paid for the rams having better mutton conformation and that were in stronger condition. However, relationships were not high, as only two of the six coefficients involving type were significant and only one of the six coefficients involving condition was significant. The three significant correlations all came from yearling groups. Type and condition scores were taken nearly 4 months before sale time. Furthermore, these traits have relatively low repeatability (Terrill *et al.*, 1950). Therefore, the correlations with price might have been much higher if the rams had been scored at sale time.

There was little or no relationship between face covering and price, as indicated by the low and non-significant correlations in all groups. However, Rambouillet rams with covered faces were superior to those with partially open or open faces for other traits, including grease and clean fleece weight, staple length, body weight, type and condition, in 64 percent (42 of 66) of the comparisons possible within groups. When evaluated with the index which includes face covering in addition to the above traits, the rams with open faces were superior in overall merit to those with covered faces in half of the comparisons. This situation may have resulted from the retention for use here of the best rams with open faces. The fact that rams with open faces did not excel those with covered faces in overall merit may explain the lack of significant correlation of face covering with price in Rambouillets. Another factor is the possible change in appearance of face covering from before shearing in the spring to sale time in the fall, although the repeatability of face covering is relatively high. Targhee rams with open faces excelled those with partially covered faces in 52 percent (31 of 60) of the comparisons involving the same traits as for Rambouillets. When evaluated with the index, the Targhee rams with open faces were superior to those with partially covered faces in every group. Nearly all of the Columbia rams had open faces and therefore an important correlation with price would not be expected.

The significant correlation coefficients between price and the selection index in four of the six groups indicate that, at least to a certain extent, the buyers of stud rams were placing somewhat the same emphasis on production traits that is placed on them because of their relative heritability and economic importance. The fact that these correlations are fairly low indicates that more progress can be made toward emphasizing the more important traits in selection.

It is noted that negative correlations between index and price were obtained for the Columbia and Targhee mature rams. Furthermore, all of the correlations in these groups were fairly low and non-significant. This appears to be due in part at least to one ram in each group which brought an exceptionally high price, but which also had some lower production records than the other rams sold in the group. These individual rams had considerable effect on the correlations because of the small number of rams available for study.

No trend toward higher or lower correlations was found over the years in the correlation of price with the various traits as determined by examining the individual yearly coefficients for each trait for Columbia and Targhee yearling rams.

Among mature rams, age of ram in years did not have a significant correlation with price. In Targhee and Rambouillet mature ram groups the younger rams brought slightly higher prices. Of the mature rams sold of all three breeds, 70 were 2 years old, 30 were 3 years old, 9 were 4 years old, 5 were 5 years old and 3 were 6 years old. Yearling rams outsold comparable groups of mature rams only half of the time. Thus, it appears that age in years was not an important factor in the price of single stud rams.

The age in days of yearling rams did not have a significant relationship to sale price. There was no consistent relationship of age of dam of yearling rams with sale price.

Type of birth (single or twin) had a significant effect on prices of yearling Columbia rams but not on yearling Targhees. Average prices for Columbia rams were $261.49, $224.64, and $131.48 for singles, twins raised as singles and twins raised as twins, respectively. Corresponding prices for yearling Targhees were $227.22, $271.36 and $225.24, respectively. Similar comparisons were not made for Rambouillet yearlings or for mature rams because many of the years did not have all types represented. It seems likely that the relation of type of birth to price of yearling Columbia rams is due to the effect of type of birth on body weight and other traits as shown by Terrill *et al.* (1948a). Type of birth should probably be indicated

in the sale catalogue so that it could be considered by the prospective purchaser.

There was no evidence of any real difference between the prices of rams with scurs and those that were strictly polled. Strictly polled rams brought higher prices than rams with scurs just half of the years for Columbia mature rams, Targhee yearling rams and Targhee mature rams. Strictly polled Columbia yearling rams brought higher prices than rams with scurs in 5 of the 6 years. The average price was slightly higher for strictly polled rams in two of the four groups. The proportions of rams with scurs were 29 percent for Columbias and 54 percent for Targhees. The scurs present on single stud rams were generally small and were not noticeable above several months growth of wool, although some of the scurs were solid.

Comparisons of comparable groups of horned and polled Rambouillets were available for yearling and mature rams in 1950 and 1951. In four of five cases the polled rams sold for higher prices than horned rams. The average advantage of the polled rams was $72.49 per head.

Comparisons of prices of registered and unregistered Rambouillets of comparable groups were available for yearlings in 1950 and 1951. In two of three cases (table 3) the registered rams brought higher prices (average of $166.11) than the unregistered rams (average of $139.79) in spite of the fact that the latter groups had higher merit as shown by their average indexes.

Many factors undoubtedly enter into the price that is paid for a ram at an auction sale other than the merit of the ram. The personalities and friendships of the buyers, as well as other psychological effects that cannot be measured, probably affect their competition for rams. Relationships in this study may be low because some of the variability among rams is removed in selecting those to be sold as singles. It is noted that the standard deviations presented in tables 1, 2 and 3 are generally lower than those presented for yearling rams of the three breeds by Terrill *et al.* (1948a, b). Furthermore, the selection of stud rams involves consideration of several traits, each of which have some importance. Of course, as the number of important traits which make up merit increase, the attention that can be paid to each trait decreases. Therefore, it was expected that the relation of sale price to individual production traits would be low.

The results presented indicate that buyers of stud rams attach real importance to production traits such as body weight, fleece weight and mutton conformation. The presentation of more information on

sale rams, particularly for those traits which are difficult to appraise visually, is probably desirable. It also appears desirable to present a selection index for each sale ram so that the buyer can take full advantage of the available records on each ram.

Summary

Relationships between sale price of single stud rams and various measures of merit were studied for rams sold at public auction at the U. S. Sheep Experiment Station and Western Sheep Breeding Laboratory, Dubois, Idaho, from 1946 to 1951. A total of 202 Columbia, 104 Targhee and 59 Rambouillet rams were included in the study.

Highest correlations for yearling rams were obtained between sale price and body weight. These correlation coefficients ranged from 0.24 to 0.73. Significant correlations with price were also obtained for order of sale, grease fleece weight, clean fleece weight, type score, condition score and index. Low and non-significant correlations with price were obtained for staple length, fleece fineness and face covering. The low correlation of price with face covering in Rambouillets may be due to differences in merit for other traits and to possible change in face covering from scoring time to sale time.

Type of birth had a significant effect on prices of yearling Columbia rams but not on prices of yearling Targhees. Age in days of yearling rams, age in years of mature rams and age of dam had no consistent relationship with price.

There was no evidence of any real difference between the prices of Columbia and Targhee rams with scurs and those which were strictly polled. Polled Rambouillet rams sold for higher prices than horned rams in four of five cases. The average advantage of the polled rams was $72.49 per head.

Registered Rambouillet rams brought higher prices than unregistered rams in two of three cases. The average advantage of the registered rams was $26.32 per head.

The results indicate that buyers of stud rams attach real importance to production traits such as body weight, fleece weight and mutton conformation. It appears desirable to present more information on sale rams, particularly for those traits which are difficult to appraise visually. It also seems advisable to present a selection index for each sale ram so that the buyer can take full advantage of the available records on each ram.

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HERITABILITY OF FUR CHARACTERS AND BIRTH WEIGHT IN KARAKUL LAMBS[1]

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KARAKUL sheep are raised chiefly for Persian lambskin fur which is popularly used for manufacturing ladies' coats. The characters of the lambskin fur, such as type, tightness, and size of curl, luster, pattern, texture and general desirability of fur, as well as hair length, are the factors which contribute to the natural beauty of the fur and affect the market price of its products. Birth weight of the lambs is also important because it influences the size of the pelt which, in turn, has a direct influence on the pelt price (Yao, Simmons and Schott, 1953).

The objective of the Karakul sheep breeders is to produce lambskins of high fur quality and large size. The extent to which fur characters can be improved is of primary importance to the breeders. A character that shows a high heritability is in general considered as one that lends itself readily to improvement by selective breeding. Simmons and Schott (1951) studied the curl size of the Karakul lambskin fur and found that the dam-progeny regression heritability for this character was 63.8 percent. The purpose of this study was to obtain heritability estimates of eight fur characters and birth weight of Karakul lambs and to investigate the probable effectiveness of selection for improving these characters.

Material and Methods

The records of eight lamb fur characters and birth weight from 728 Karakul lambs and their 207 dams and 22 sires were used in this study. The sires were purebred Karakul rams. The dams consisted of 72 purebred Karakuls, 44 Karakul × Blackfaced Highlands, 48 Karakul × Corriedales, and 43 Karakul × Navajos. The grade ewes were of 50 to 99.22 percent Karakul breeding. The sires and dams were raised and bred at the Agricultural Research Center, Beltsville, Maryland, from 1942 through 1949. Of the lambs, 246 were purebred Karakuls, 145 Karakul × Blackfaced Highlands, 186 Karakul × Corriedales and

[1] This work was conducted jointly by the sections of Sheep and Goat Husbandry, Animal Fiber, and Fur Farming Investigations, Animal Husbandry Division, Bureau of Animal Industry, A.R.A., U.S.D.A.

151 Karakul × Navajos. The animals were recorded for their lamb fur characters and birth weight within 48 hours after birth.

The eight fur characters studied were type, tightness, and size of curl, pattern, luster, texture and general desirability of fur, and hair length. The characters were scored by a committee of three judges, using the method previously described by Schott and Simmons (1941). The average of the three judges' scores was taken as the primary observation for each character. Except for texture, the scores were in a series of 1 to 10 points. The smallest curl size, the tightest fur, the most lustrous fur, the best pattern and the most desirable type of curl was scored as 1, and the least desirable of each character was scored as 10. Texture was scored on a 5-point scale, 1 for the finest and most silky fur, and 5 for the coarsest and most wiry fur. A set of samples for each fur character was used as a standard in scoring the lambskins. Birth weight was recorded in pounds and hair length measured in millimeters.

Records on curl type, pattern texture and hair length were available on only 284 of the lambs. Fur records on both sire and dam of 673 of the lambs were available with respect to curl size, tightness and luster of fur. Similarly, data on the sire and dam of 418 of the lambs were available for pattern, desirability and birth weight.

The analytical methods of Fisher (1946) and Snedecor (1948) were used to calculate the independent variances and covariances caused by breed, sire and year differences. The methods of paternal half-sib correlation, intra-sire regression on dam and regression on mid-parent as described by Lush (1949), and the variance component method as used by Windsor and Clark (1940), were adopted in the estimation of heritability. The standard errors were obtained by using the formulas given by Hazel and Terrill (1945).

Results and Discussion

The mean variances for the nine characters of the Karakul lambs caused by the different factors, breed, sire, year and error, are listed in table 1. Both differences between sires and differences between years were significant for all the characters except pattern. This suggests that selection of better sires could improve most of the lambskin characters. Breed differences were significant for curl size, tightness, luster, desirability and birth weight. Selection of an appropriate breed of sheep as the foundation stock to be graded up is therefore important in improving these characters.

TABLE 1. MEAN VARIANCES OF THE KARAKUL LAMB CHARACTERS

Characters	No.	Breed	Sire	Year	Error
Curl size	728	21.6**	19.7**	26.9**	3.5
Tightness	728	15.9**	20.9**	49.7**	1.9
Luster	728	3.9*	33.0**	84.2**	1.3
Desirability	728	12.2**	4.7**	4.2*	1.9
Curl type	284	2.9	9.1**	18.6**	2.9
Pattern	284	5.1	2.5	5.0	2.2
Texture	284	1.2	1.4**	3.6**	0.6
Hair length	284	11.1	14.3*	28.6*	7.2
Birth weight	728	9.3*	9.5**	19.3**	3.3

* Significant at 5 percent level.
** Significant at 1 percent level.

The mean variances for six lambskin characters of dams, and the covariances between dams and lambs, caused by breed, sire, and error are listed in table 2. Breed differences were significant for all six characters. These indicated that the purebred Karakul ewes and the ewes of the three different grade groups differed significantly in these characters. When the ewes were divided into sire groups, significant differences were found in tightness and desirability of fur and birth weight. The general lamb fur desirability of dams was also significantly different in different years.

The average scores and measurements for the lamb fur characters of sires and dams were used as the scores and measurements for the characters of the mid-parent. The mean variances of six lambskin characters of the mid-parents and the covariances between mid-parents and lambs caused by breed, year and error are shown in table 3. Year differences were significant in five of the six characters. When

TABLE 2. MEAN VARIANCES OF DAMS' CHARACTERS AND THE COVARIANCES BETWEEN THE CHARACTERS OF THE DAMS AND THEIR LAMBS

		Breed		Sire		Year		Error	
Characters	No.	MV[1]	Cov.[2]	MV	Cov.	MV	Cov.	MV	Cov.
Curl size	728	85.9**	104.2	5.2	102.9	5.1	24.3	3.4	758.8
Tightness	728	91.6**	74.0	4.0**	−19.0	3.5	−37.1	1.9	233.0
Luster	728	12.6**	−5.1	2.0	−30.7	3.6	60.9	3.3	166.7
Desirability	728	149.2**	101.9	5.3**	33.5	9.8**	−2.1	1.3	80.0
Pattern	284	6.1*	16.6	2.3	−11.6	4.9	0.2	2.2	29.5
Birth weight	728	101.5**	84.1	4.2**	43.6	0.9	16.7	2.0	223.2

* Significant at 5 percent level.
** Significant at 1 percent level.
[1] MV=Mean variance.
[2] Cov.=Covariance.

TABLE 3. MEAN VARIANCES OF MID-PARENTS' CHARACTERS AND THE COVARIANCES BETWEEN THE CHARACTERS OF THE MID-PARENTS AND THOSE OF THEIR LAMBS

Characters	No.	Breed		Year		Error	
		MV [1]	Cov.[2]	MV	Cov.	MV	Cov.
Curl size	673	22.5**	48.2	14.5**	49.4	1.7	390.4
Tightness	673	22.0**	29.3	27.1**	219.0	1.0	103.6
Luster	673	1.5	4.1	3.0**	−6.1	0.7	184.1
Pattern	418	15.2**	15.2	5.5**	−9.6	0.9	−9.5
Desirability	418	17.1**	22.4	2.0*	−5.5	0.7	30.0
Birth weight	418	12.1**	11.5	1.4	14.4	1.1	110.8

* Significant at 5 percent level.
** Significant at 1 percent level.
[1] MV=Mean variance.
[2] Cov.=Covariance.

the year influence in this table was compared with that in table 2, it showed that in the mid-parent records there were more significant year differences than in the dam records. The covariance between mid-parents and lambs for the pattern of fur was negative. It indicated that this character had a negative correlation between the two generations or that the effect of environmental differences disturbed the resemblance between the two generations on this particular character.

The heritability estimates for the nine lambskin characters estimated by three different methods are listed in table 4. The half-sib correlation method is based on the variances occurring in the characters of the lambs alone. The coefficients represent the portion of the variances in the characters caused by the genetic influence of the sires.

TABLE 4. HERITABILITY ESTIMATES FOR FUR CHARACTERS AND BIRTH WEIGHT OF KARAKUL LAMBS

Characters	Half-sib Method		Intra-sire Regression		Mid-parent Regression	
	Herita-bility	S.D.	Herita-bility	S.D.	Herita-bility	S.D.
Curl size	43.1	18.0	67.6	7.6	36.0	7.7
Tightness	60.2	27.7	37.7	7.7	16.5	8.1
Luster	(150.2)	26.4	15.2	4.8	44.3	8.9
Desirability	17.7	8.6	19.4	9.3	10.1	8.0
Curl type	25.2	16.1	....	....	....	....
Pattern	2.0	7.6	10.4	12.2	0	0
Texture	13.1	11.8	....	....	....	....
Hair length	9.2	10.3	....	....	....	....
Birth weight	17.7	9.4	34.5	9.8	25.4	8.8

The intra-sire regression method is based on the covariances between the lambs and dams, and the dams' variances. The estimates indicate how much the variances of the dams' characters would affect the variances in the lambs' characters. The mid-parent regression method is based on the covariances between lambs and mid-parents, and the mid-parent variances. The heritability estimates indicate how much the variances of the mid-parent characters would influence the variances of the lambs' characters. The three methods had different bases in calculation and each had its own importance in the estimation of heritability.

The heritability estimates obtained by the half-sib correlation method showed that tightness of fur had the highest heritability, 60.2, and pattern had the lowest, 2.1. Luster had an estimate higher than 100 percent. There must be certain kinds of factors which caused this extremely high estimate. The sampling methods and the scoring technique were possible sources and might be responsible for the extremity. A few genes which might possess the epistatic or dominant effects in certain combination and under certain conditions, and were transmitted from a part of the sires, might also be sources for the high variances between sires and bring the estimate up to more than 100 percent. For the coefficients calculated by the intra-sire regression on the dam, curl size had the highest heritability estimate, 67.7, and pattern the lowest, 10.4. Simmons and Schott (1951) obtained a similar heritability estimate of 63.8 percent for curl size with a part of the same data used in this study. For the coefficients from the regression on mid-parent method, luster had the highest estimate, 44.3, and pattern had zero heritability. The results from the three methods showed that three of the nine characters had high heritability—curl size, tightness and luster, three had medium heritability—desirability, curl type and birth weight, and three had low heritability—pattern, texture and hair length.

The heritability estimates for five characters computed separately in purebred Karakuls and three grade groups are listed in table 5. For the size of curl, purebred Karakul had the highest heritability by the half-sib method, 59.7, while Karakul $\times$ Navajo had the lowest, 6.6. By the intra-sire regression method Karakul $\times$ Blackfaced Highlands showed the highest heritability, 55.9, in curl size, except that Karakul $\times$ Navajo here had a heritability of more than 100. This possibly was another case caused by the sampling methods, the scoring technique, or a few epistatic or dominant genes transmitted from a part of the Karakul $\times$ Navajo dams. However, these factors probably

lowered the heritability estimates from the half-sib correlation method. The estimates from mid-parent regression method had figures similar to those from intra-sire regression method.

For the other characters, purebred Karakuls had zero heritability for the tightness of fur character by the regression methods, while the Karakul × Blackfaced Highland had the highest estimates by all three methods (67.6, 72.0 and 68.1). For luster of fur, the Karakul × Corriedale had a heritability of 99.2 by the half-sib method, an unusually high estimate perhaps caused by the factors mentioned above. These factors probably lowered the heritability estimates by the regression methods. The trend was contrary to that occurring in curl size in Karakul × Navajo. For the general desirability of fur, purebred Karakuls showed zero heritability by the regression method. Similarly, the Karakul × Blackfaced Highland showed zero heritability by the half-sib method. For the birth weight, purebred Karakul had the highest coefficient, 63.8, by the regression method, and the Karakul × Navajo showed the highest coefficient, 51.3, by the half-sib method.

All the estimates from the mid-parent regression method were similar to those from the intra-sire regression method for the different breeding groups (table 5). It is probable that the phenotypic variance of sires when combined with the dams' variances within different breeding groups may not have affected the heritability estimates. The similarity of estimates by these two methods may also be due to the small number of sires and the small phenotypic variances in sires which were not large enough to make the combined average of dams and sires much different from the variance of the dams alone.

It was also observed that different breeding groups had different estimates of heritability for different characters. It may be assumed that the difference in genetic constitution and the presence or absence of the factors which had some connection with the sires or dams made the estimates by the half-sib method or regression method increase so much as to exceed 100 percent. The factors which had some connection with the dams might lower the heritability estimates by the half-sib method. On the other hand, if these factors were connected in some way with the sires, the heritability estimates by the regression method would be lowered. The heritability estimate by the half-sib method for luster (table 4) was more than 100 percent, while the estimate by the intra-sire regression method was only 15.2.

The heritability estimate by the regression method for curl size in the Karakul × Navajo (table 5) was more than 100 percent while that by the half-sib correlation method was only 6.6.

The probable rate of change which may result from selection in the eight lamb fur characters and birth weight is determined partly by their heritabilities. Curl size, tightness and luster in the study were

TABLE 5. HERITABILITY ESTIMATES FOR FUR CHARACTERS AND BIRTH WEIGHT IN KARAKUL LAMBS OF PURE AND GRADE BREEDING

	Half-Sib Method		Intra-Sire Regression		Mid-Parent Regression	
	Heritability	S.D.	Heritability	S.D.	Heritability	S.D.
Curl size:						
Karakul	59.7	24.6	40.6	12.5	42.4	14.0
Karakul × Blackfaced Highland	29.7	26.0	55.9	18.3	54.6	19.1
Karakul × Corriedale	51.4	26.1	32.7	19.1	30.9	17.7
Karakul × Navajo	6.6	20.4	(114.6)	16.1	(114.6)	17.0
Tightness:						
Karakul	45.7	21.9	0	0	0	0
Karakul × Blackfaced Highland	67.6	32.2	72.0	16.9	68.1	17.3
Karakul × Corriedale	65.0	28.3	24.1	14.2	22.7	15.2
Karakul × Navajo	13.3	22.0	31.2	22.3	37.8	24.0
Luster:						
Karakul	66.0	25.5	75.8	15.1	81.7	17.9
Karakul × Blackfaced Highland	44.7	28.7	38.1	14.6	40.4	15.4
Karakul × Corriedale	(99.2)	32.6	12.7	16.6	5.9	18.2
Karakul × Navajo	77.4	33.5	42.4	18.2	42.6	9.7
Desirability:						
Karakul	9.4	13.7	0	0	....	....
Karakul × Blackfaced Highland	0	0	31.3	20.6	....	....
Karakul × Corriedale	12.8	18.4	6.7	20.4	....	....
Karakul × Navajo	15.2	22.4	11.4	17.4	....	....
Birth weight:						
Karakul	15.4	15.2	63.8	16.4	....	....
Karakul × Blackfaced Highland	0	0	17.7	20.0	....	....
Karakul × Corriedale	6.4	16.9	34.2	24.1	....	....
Karakul × Navajo	51.3	29.5	22.9	22.8	....	....

of high heritability, indicating that these characters can be improved by selection and breeding practices. Desirability, curl type and birth weight might have some possibility of improvement by merely selecting the better scored animals for breeding stock. Pattern, texture and hair length would be capable of little or no improvement through mass selection unless line selection and cross breeding were practiced to obtain better sets of gene combinations.

For the different breeding groups, the estimates show that the

purebred Karakuls would be capable of the greatest improvement in curl size, while Karakul × Blackfaced Highland and Karakul × Corriedale might also give good results in selection of curl size. Improvement in the tightness of fur could be readily obtained in the Karakul × Blackfaced Highlands and also in the Karakul × Corriedales. Luster of fur and birth weight could be improved most effectively in the Karakul and Karakul × Navajo groups.

Summary

The records of eight lamb fur characters and birth weight from 728 purebred and grade Karakul lambs and their 207 dams and 22 sires raised at the Agricultural Research Center, Beltsville, Maryland, from 1942 through 1949, were used to calculate heritability estimates. The estimates were also computed separately in the purebred and three grade groups—Karakul × Blackfaced Highland, Karakul × Corriedale and Karakul × Navajo. Three methods—paternal half-sib correlation, intra-sire regression on dam, and mid-parent regression—were used in the estimation. The variances for lambs, dams and mid-parents and the covariances between lambs and dams, and between lambs and mid-parents were corrected with the variances and covariances caused by breed, year and sire differences.

The nine characters were divided into three heritability groups: high heritability group—curl size (36.0—67.6), tightness (16.5—60.2), luster (15.2—44.3); medium heritability group—desirability (10.1—17.7), curl type (25.2), birth weight (17.7—34.5); and low heritability group—pattern (2.0—10.4), texture (13.1), and hair length (9.2). Different breeding groups had different heritability estimates. Purebred Karakuls had the highest heritability in curl size (59.7) with half-sib method, in luster (75.8) and birth weight (63.8) with intra-sire regression method, and again in luster (81.7) with mid-parent regression method. The Karakul × Blackfaced Highlands had the highest heritability in tightness (67.6, 72.0, 68.1) by all three methods, in curl size (55.9, 54.6) with both regression methods, and in desirability (31.3) with the intra-sire regression method. The Karakul × Navajo had the highest heritability only in birth weight (51.3) with the half-sib method. The characters which had high heritability could be expected to give the most response in selection, while the characters with low heritability may be improved when line selection and cross breeding systems are practiced.

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SKIN FOLDS IN RAMBOUILLET SHEEP

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THE desirability and extent of skin folds on fine wool sheep is a controversial and much discussed question. Stud breeders disagree on how far it is wise to proceed toward extreme smoothness or plainness of body. An answer to this question is desired in order to eliminate confusion among sheep breeders of the range states.

Assuming equal production, smooth sheep are preferred to wrinkled sheep because of ease of shearing, fewer shear wounds, more uniform fleece with fewer second cuts, less blowfly strike and more desirable market appearance of the lambs. It is common belief that a certain number of wrinkles are necessary in order to maintain maximum production of quality wool. Jones *et al.* (1944) compared B and C type Rambouillet sheep and found the B type (wrinkled) to produce approximately 1 pound more grease wool and the C type (smooth) to have a slight advantage in staple length. Spencer, Hardy and Brandon (1928) found that grease wool increased as the number and size of skin folds on the neck increased. Neither of these groups of workers reported any important differences in fineness as related to skin folds. The work of Carter (1941) and Madsen, Esplin and Phillips (1943) indicates that the number of skin folds may vary with age and level of feeding, but in all cases there was a high correlation of skin fold values between different ages and sizes. Evidence that skin folds are hereditary and may be controlled by selection can be ascertained from the work of Jones *et al.* (1946) and Terrill and Hazel (1943, 1946) who reported heritability factors of 0.45, 0.32 and 0.39, respectively.

Two projects are in progress at the Sonora Station which shed some light on the question of skin folds in fine wool sheep.

Breeding Project

In 1946, the Rambouillet breeding flock of this Station,[1] consisting of about 200 ewes, was separated into two groups based on number of skin folds. These ewes have been managed as one group in accordance

[1] Tex. Agr. Expt. Sta. Substation 14, Sonora, Texas.

with good husbandry practices, but they have been bred as two distinct groups. These two groups will be referred to hereafter as the smooth group and the folded group (those having skin folds intermediate in size and number). The skin fold values were taken in a manner outlined in detail by Jones *et al.* (1946). Briefly, the values represent a total score for each animal based on the number, size and location of individual folds. The higher the score, the greater the amount of skin folds. At the start the ewes were divided with those of a skin fold value of 33 or below going into the smooth group and those above 33 going into the folded group. At the outset the smooth group of ewes had an average skin fold value of 20.1 as compared to 52.4 for the folded group. In the years since 1946 selection has tended to favor the smooth sheep in both groups, and as a result the skin fold values have decreased in both groups. At present the smooth group has relatively smooth bodies and the folded group is comparable to a Medium C type of a few years back.

The data collected included weaning weight, staple length, body score, face covering and skin fold values on the lambs. Face covering scores used were as follows: Open (1), Medium (2), Covered (3), Wool Blind (4).

The ram lambs were removed from the flock at weaning and the better ones put on a feeding test, with the remainder going to market. The ewe lambs were run on the range over winter; in the spring they were weighed, measured for staple length (average of side, shoulder and thigh measurement), shorn and the fleeces scoured individually to obtain clean wool production. Skin fold records on the yearlings were taken out of the wool. On the mature ewes the only records taken were grease wool and staple length, except at the 1952 shearing season when a random sample of one fleece in 10 was taken from each group and scoured to obtain scouring data on the mature ewes.

Results

The data collected on the mature ewes are presented in table 1, and followed by an analysis of variance in table 2. These data show a highly significant difference favoring the smooth sheep for staple length, and using the interaction mean square for testing, there is a significant difference favoring the folded sheep for grease wool. The samples scoured in 1952 gave a yield of 44.4 percent for the folded group and 50.4 percent for the smooth. This difference in shrinkage seems large and would more than erase the small amount of additional

TABLE 1. AVERAGE PRODUCTION OF MATURE EWES, 365-DAY BASIS

Year	Type	No.	Grease Wool	Staple Length
			lb.	cm.
1946	Smooth	83	10.11	6.62
	Folded	78	10.65	6.20
1947	Smooth	86	9.53	....
	Folded	74	9.62	....
1948	Smooth	72	8.45	5.94
	Folded	58	8.44	5.63
1949	Smooth	100	8.03	6.67
	Folded	65	8.83	6.38
1950	Smooth	88	9.00	6.66
	Folded	66	9.38	6.16
1951	Smooth	83	8.69	7.32
	Folded	78	9.92	6.88
1952	Smooth	109	8.43	7.33
	Folded	80	8.71	6.84
Overall aver.[1]	Smooth	621	8.86	6.80
	Folded	499	9.41	6.39

[1] The number included in the totals for staple length does not include the 160 ewes of the 1947 season.

grease wool favoring the folded sheep. The additional grease wool produced by the folded group is largely a matter of grease and dirt held in the protected area of the folds, and the tighter fleece of the folded ewes. Although no definite data on this point were collected, most of the folded ewes appeared to have greater fleece density. The reason for this is not entirely clear, but it does not appear that the reason is necessarily physiological, since some smooth ewes have tight fleeces. The most plausible explanation is genetic linkage. Most fine wool sheep in years past have been heavily folded, short stapled and

TABLE 2. MEAN SQUARES FOR GREASE WOOL AND STAPLE LENGTH OF MATURE EWES

Source of Variance	Grease Wool		Staple Length	
	Degrees of Freedom	Mean Square	Degrees of Freedom	Mean Square
Years	6	82.70**	5	36.97**
Type	1	58.80**	1	40.26**
Interaction	6	7.13**	5	0.31
Error term	1106	2.06	948	0.82

** Significant at the 1% level of probability.

tight fleeced. In recent times great selection pressure has been exerted toward long staple and smoothness simultaneously. With a limitation on clean wool production due to the level of nutrition or the productive capacity of the sheep, selection for staple length automatically results in selection for an open type of fleece.

The weaning data and their analysis are presented in tables 3 and

TABLE 3. WEANING RECORD ON LAMBS[1]

Year	Type	No.	Skin Folds	Staple Length (cm.)	Weaning Weight (lb.)	Face Covering	Conformation Score
				Rams			
1946	Smooth	24	22.83	3.65	86.86	2.71	76.33
	Folded	27	42.48	3.33	92.96	2.63	74.26
1947	Smooth	31	13.10	3.69	82.97	2.87	78.13
	Folded	33	46.87	3.55	81.48	3.21	73.61
1948	Smooth	32	11.87	4.50	91.97	2.63	76.50
	Folded	22	33.86	3.81	83.54	2.55	73.09
1949	Smooth	35	....	4.25	85.08	2.77	78.71
	Folded	27	....	3.80	84.37	2.81	76.15
1950	Smooth	39	9.41	4.67	88.09	2.76	79.54
	Folded	36	25.00	4.09	82.30	2.69	74.69
1951	Smooth	39	3.64	4.33	73.72	2.54	79.38
	Folded	38	16.68	3.64	73.29	2.66	76.47
Aver.	Smooth	200	11.17	4.23	84.45	2.71	78.28
	Folded	183	30.32	3.71	82.31	2.77	74.82
				Ewes			
1946	Smooth	27	15.04	3.88	85.37	2.37	80.22
	Folded	26	31.04	3.67	89.00	2.46	77.65
1947	Smooth	52	15.56	3.90	75.13	2.73	79.69
	Folded	42	36.55	3.67	71.05	3.09	74.29
1948	Smooth	32	10.34	4.77	88.28	2.28	78.53
	Folded	22	28.64	4.04	80.27	2.77	73.47
1949	Smooth	35	....	4.53	80.00	2.43	80.86
	Folded	25	....	3.99	77.53	2.84	79.16
1950	Smooth	52	5.94	4.80	79.02	2.24	79.84
	Folded	35	16.40	4.29	74.71	2.61	79.06
1951	Smooth	35	3.51	4.45	70.97	2.37	79.60
	Folded	36	10.22	3.97	68.80	2.77	78.58
Aver.	Smooth	233	9.99	3.71	79.09	2.42	79.79
	Folded	186	24.31	3.39	75.77	2.78	77.05

[1] Staple length and body weight adjusted to 182.5 days.

4. The rams have significantly higher skin fold values than the ewes. Skin fold values tend to increase as the animal develops, especially in those on a high plane of nutrition. The skin fold values on yearlings were about 34.5 percent higher than the lamb values. However, the number of folds on the lambs is a reliable value to use in selection, since it is highly correlated ($r=0.81$) with that as a yearling. A large part of the disparity between the two records can be attributed to the

TABLE 4. MEAN SQUARES FOR WEANING DATA ON LAMBS

Source of Variance	Degrees of Freedom	Staple Length	Weaning Weight	Face Covering	Conformation Score
Years	5	16.000**	4919.00**	4.560**	250.46**
Within years	796	0.348	312.26	0.732	39.03
Sexes within years	6	1.937**	1283.67**	1.987*	151.12**
Within sex and year	790	0.336	304.88	0.722	38.18
Types within sex and year	12	3.832**	335.78	2.215**	266.16**
Within type, sex and year	778	0.282	304.44	0.699	34.66

* Significant at the 5% level.
** Significant at the 1% level.

human element in ascertaining the size of the folds. The folded group has a slightly but significantly greater face covering. It would appear that this is due to genetic composition of the population and not necessarily to any physiological reason. Most of the folded rams used had more covering on the face. The smooth group of lambs had a longer staple, larger size at weaning, and higher type score. It is entirely possible that the low body score for the folded group was due in part to discrimination against folds in scoring. In this study the smooth lambs weighed more at weaning, but caution should be used in applying this knowledge to other flocks of sheep, or to the sheep population as a whole. The size of the flock used has been small, and one outstandingly good ram and his offspring used for a number of years in the smooth group were probably responsible for

TABLE 5. SPRING RECORDS ON YEARLING EWES [1]

Year	Type	No.	Skin Folds	Grease Weight (lb.)	Clean Weight (lb.)	Staple Length (cm.)	Body Weight (lb.)
1947	Smooth	26	31.39	6.93	3.52	7.18	83.17
	Folded	24	46.95	7.23	3.59	5.98	75.00
1948	Smooth	36	26.61	6.66	3.25	7.00	73.00
	Folded	15	45.46	6.75	3.11	6.75	63.53
1949	Smooth	29	24.62	6.93	3.52	7.18	83.17
	Folded	20	51.50	6.81	3.19	5.95	77.90
1950	Smooth	33	19.52	6.78	3.46	7.16	88.03
	Folded	23	43.17	6.56	3.11	6.21	83.48
1951	Smooth	49	5.84	6.96	3.24	7.30	71.08
	Folded	35	21.11	6.95	3.06	6.54	66.71
1952	Smooth	36	8.39	6.06	3.05	7.54	72.16
	Folded	36	16.90	6.22	2.85	6.58	68.13
Aver.	Smooth	209	17.80	6.72	3.32	7.23	77.45
	Folded	153	33.85	6.73	3.12	6.35	72.02

[1] Wool production and staple length are corrected to a 365-day basis except for 1952, when the actual records were used.

this rather than any influence of folds on rate of growth. It seems to be a well established fact that the smooth sheep produce wool of a longer staple. The explanation appears to be the simple fact that, since the production of the ewes is limited by level of nutrition or productive capacity of the ewe, distribution of the wool over a larger area, as with the folded sheep, would invariably result in wool of shorter staple.

Both year and sex had a highly significant influence on all the factors studied. That sex would do so is understandable. The variability of years is due to variable climatic conditions and to the fact that the rams used were not the same each year.

TABLE 6. MEAN SQUARES OF DATA ON YEARLING EWES

Source of Variance	Degrees of Freedom	Grease Weight	Clean Weight	Staple Length	Body Weight
Years	5	9.71**	2.58**	11.3*	2861.0**
Types	1	0.00	0.346	54.0**	1585.0**
Interaction (year × type)	5	0.497	0.022	1.56*	80.0
Within type and year	350	0.971	0.175	0.659	49.9

* Significant at the 5% level.
** Significant at the 1% level.

Averages by years and an analysis of the data on the yearling ewes are presented in tables 5 and 6. These data do not show a significant difference between types for grease and clean wool production. There is a significant difference in body weight and staple length in favor of the smooth group. The same explanation may be applied to yearling weights as was given for the lambs, and this additional size of the smooth yearlings is likely a contributing factor to their greater wool production. With respect to grease wool production, the yearling ewes do not react in the same manner to type as do the mature ewes.

Ram Progeny and Performance Testing Project

In this project the more promising ram lambs at weaning are selected, and with those of other breeders, are sheared and put on a feeding test, the purpose of which is to facilitate and improve the accuracy in stud ram selection. During the test detailed records are taken on wool production, rate of gain, body type (including skin fold values) and other characters of less importance. In the past 4 years 441 Rambouillet rams have been fed according to appetite on a growing

TABLE 7. CORRELATION COEFFICIENTS FOR SKIN FOLD VALUES WITH DAILY GAIN AND FLEECE CHARACTERISTICS

	Daily Gain	Clean Wool	Grease Wool	Staple Length	Yield
Final skin folds gross correlation	0.19**	0.24**	0.41**	—0.22**	—0.22**
Final skin folds within sire groups	0.00	0.25**	0.47**	—0.11*	—0.18**
Initial skin folds (gross)	0.13*	0.13*	0.41**	—0.23**	—0.20**
Increase skin folds (gross)	0.15*	0.30**	0.30**	—0.13*	—0.16**

* Significant at the 5% level.
** Significant at the 1% level.

ration consisting of whole oats and alfalfa hay. In the first 2 years the feeding periods were 308 and 277 days respectively, and in the last 2 years 224 days. Records on wool production were converted to a 365-day basis.

Correlation coefficients of skin fold values with the various productive characters are given in table 7, and the relationship of skin folds to daily gain and wool production is shown graphically in figure 1. In 3 of the 4 years' work skin fold records were taken at the start of the test, and in all 4 years they were taken at the end of the test. In the first 3 years it is possible to break down the final skin fold values into the two parts: initial folds and increase in folds during

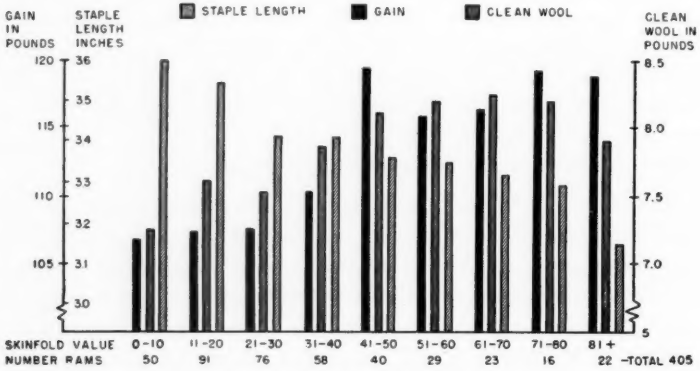

Figure 1. Effect of skin folds on productivity of yearling rams.

the test. The average increase during the test is approximately 169 percent or the equivalent of two large neck folds. This increase is considered to be higher than it would be for animals developed more slowly over a longer period of time.

Final skin fold values show a highly significant positive correlation with rate of gain and wool production and a highly significant negative correlation with staple length and yield. The relationship indicated by the significant but low correlation values may be due to either genetic or physiological causes. The correlation between skin folds and daily gain lies largely between sire groups, and even this is reduced when the additional wool and heavier shrinkage of the folded sheep are removed ($r=0.15$). In no case is the correlation value dealing with rate of gain sufficiently high to have any real predictive value. The negative correlation of skin folds with staple length and yield are as would be expected. The association between skin folds and productivity can perhaps be explained by one or a combination of the reasons listed below.

1. Under the conditions of this test skin folds with the additional skin surface and wool producing follicles enable the sheep to produce a greater amount of wool.

2. Due to the prevalence of skin folds in early Rambouillet sheep, a certain amount of linkage still exists between skin folds and productivity.

3. Since skin folds increase as the animal grows and develops, there would be a tendency for those doing the best to also develop more folds. This would result in an association between skin folds and productivity.

The importance of this latter reasoning is pointed out by the relative importance of the two components, initial folds and increase in skin folds. The former is less important in so far as it influences wool production and rate of gain. The fact that the correlation coefficient for wool production is greater within sire groups than the gross correlation may be explained by this reasoning. Many breeders have experienced this association in that in the better years their lambs carry more skin folds.

There are a number of individuals among the tested smooth rams that are among the top producers. This suggests that with selection pressure exerted for gaining ability and clean wool production along with smoothness it may be possible to eliminate excessive skin folds without sacrificing production.

Discussion

Data presented on the ewe flock show no advantage for skin folds save for approximately one-half pound more grease wool. This supports the idea held by many breeders that a certain number of skin folds is necessary. It cannot be denied that breeders still sell grease wool, and, according to current methods of marketing, it is unlikely that there would be a great difference in price of the wool produced by the two groups of ewes. This would possibly result in slightly greater income from the wool of folded sheep. The folded sheep appear less favorable with respect to the other characters studied—especially staple length.

The data presented for the rams appear to contradict that collected on mature ewes, particularly in wool production. This different reaction of the two sexes appears to be a common occurrence, since in the group of mature stud rams carried by the station the folded rams are heavier producers.

A closer study of the data indicates that sex is not the primary cause of this discrepancy, but instead the level of nutrition appears to be the controlling factor. The rams are carried on a high plane of nutrition and the ewes have been on a low plane of nutrition. The average flock of range ewes is on a low plane of nutrition even under average to good range conditions, when we consider the job they are expected to do (i.e., develop and maintain a large body, provide energy for utilization of range, produce a heavy clip of desirable wool, and produce at weaning time a heavy lamb). In the ewe flock on the range the limiting factor would appear to be nutrients available for wool production, and not skin surface area. Supporting evidence for this theory is the highly significant interaction between type and year in the case of the mature ewes. The difference in favor of the folded group was greater in the more favorable years. In the case of the rams there is more feed available with fewer outlets for the nutrients, resulting in slightly more wool production by the folded group. This explanation would appear to fit in with a realistic approach to the reason why early breeders developed the heavy folded sheep. First of all, early breeders responsible for the development of skin folds were located in the East in more favorable grazing areas, and greater emphasis was placed on wool as compared to lamb production. Folded sheep would have an advantage under these conditions, especially in the case of the rams where most selection pressure would be exerted. Proper credit is not given to the early

breeders in saying that they favored wrinkles only on the belief that they were associated with higher production.

There remains the fact that the small advantage in grease wool for the folded sheep in a commercial ewe flock is unimportant compared to the other advantages favoring the smooth sheep. Since fine wool sheep production today is, for the most part, relegated to the less favorable agricultural areas, and in light of deteriorating range conditions, it is doubtful that conditions will again favor sheep with a greater number of wrinkles. Whether justly or not, the preference of feeders and packers for smooth lambs is commonplace as indicated by their discrimination against skinfolds. In view of the fact that about two-thirds of the breeders' income is from lambs, it is understandable why they favor smooth sheep. On this basis it appears that our present course away from heavily wrinkled sheep is a wise one, although selection pressure against folds should be based on their economic importance instead of personal prejudice.

Summary

1. A flock of approximately 200 ewes was divided into two groups based on number of skin folds, bred within their respective groups and compared in production over a period of 6 years.

2. In this study the folded ewes produced approximately one-half pound more grease wool as mature ewes, but the smooth ewes had an advantage on all other characters studied. The smooth group produced significantly greater staple length, more weight and higher body score at weaning and were heavier as yearlings.

3. The performance of ram lambs on feed was compared, according to the number of skin folds, with the folded rams showing a slight advantage in gain and wool production, but producing shorter staple wool.

4. An attempt was made to reconcile these two apparently contrasting viewpoints.

5. In view of the many advantages of smooth sheep, along with their preference by the industry, breeders can no longer justify skin folds for the slight additional grease wool.

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A STUDY OF SHEEP FED HIGH LEVELS OF POTASSIUM BICARBONATE WITH PARTICULAR REFERENCE TO INDUCED HYPOMAGNESEMIA[1]

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THE characteristic symptoms of grass tetany have suggested an underlying alteration in mineral metabolism. This disease, reported in many parts of the world, appears to be characterized by a hypomagnesemia and sometimes by hypocalcemia. The literature on this subject has been reviewed by Russell (1944). The malady termed "wheat pasture poisoning" which occurs in the High Plains region of the southwestern United States (McMillen and Langham, 1942; Pearson *et al.*, 1949) appears to be characteristic of grass tetany.

In the study of the etiology of wheat pasture poisoning, the high potassium content of the wheat plant has been suspected as a contributory factor (Pearson *et al.*, 1949; Odell *et al.*, 1952). In the course of studies at this Station, it has been observed that the young wheat plant from areas where this disease is enzootic is very high in potassium containing in the order of 3.5 percent on a dry basis (Pearson *et al.*, 1949).

Although some of the experimental results have indicated no effect of high dietary levels of potassium, still other findings cast doubt on the finality of these observations. Pearson *et al.* (1949) fed a ration containing 5 percent potassium as the bicarbonate to adult ewes without apparent effect on the health of the animal or significant alteration of the amounts of calcium, magnesium or potassium in the blood serum. Similar results have been reported recently by Odell *et al.* (1952) who observed no adverse effects from administration of about 7.7 percent potassium chloride in the diet or 116 gm. potassium chloride per head per day in drinking water while on pasture.

On the other hand, the toxicity of potassium salts administered by stomach tube to rats has been established (Thatcher and Radike, 1947). Pearson (1948) and Colby and Frye (1951) reported a

[1] This work was supported in part by a grant from the Dow Chemical Company, Freeport, Texas, administered through the Texas A. and M. Research Foundation.

[2] The authors are deeply indebted to Raymond Fowler and Richard W. Moore for assistance in the care and management of experimental animals, and to Dr. R. D. Turk and Dr. W. S. Monlux of the Department of Veterinary Science for the histological examinations

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depressed rate of gain in rats when potassium was fed at the 5 percent level as the bicarbonate or carbonate. A relationship between potassium and magnesium metabolism has also been indicated by Smith (1949) in work with dogs. Smith reported a drop in serum potassium coincident with the injection of magnesium sulfate. Pearson (1948) reported a hyperemia similar to that of magnesium deficiency in a few animals fed high potassium diets although total blood magnesium was not altered significantly. Colby and Frye (1951) demonstrated a reduction in serum magnesium in the rat by feeding high levels of potassium bicarbonate. Although no reduction in serum magnesium was noted, data obtained in a balance study with sheep recently reported by Eaton and Avampato (1952) indicated that feeding potassium chloride to give a total of 3.2 percent potassium in the diet reduced the retention of both calcium and magnesium.

The present paper reports the results of experiments in which there was a further attempt to demonstrate potassium toxicity in sheep when potassium bicarbonate was included at a high level in a natural diet. The studies were designed also to ascertain a possible synergism between a low water or a low salt (NaCl) supply and a high potassium intake, and to determine the possible effects of *ad libitum* supplementation of inorganic magnesium on potassium toxicity. In previous studies (Colby and Burns, 1951) in which 3.5—3.8 percent potassium was fed to sheep, there has been observed a marked increase in salt and water intake by the sheep fed the added potassium. A physiological interrelationship between potassium and sodium has been shown by Conway and Hingerty (1948).

Experimental

Thirty adult Rambouillet ewes weighing from 70–100 pounds were used in this study. To insure maximum consumption of potassium, a single mixed diet was fed. The basal diet consisted of cottonseed hulls —50 percent, ground milo grain —30, and soybean oil meal —20. The soybean oil meal was replaced in the sixth week of experiment by milo gluten meal. This diet was calculated to contain 0.15 percent calcium, 0.25 percent phosphorus and 0.20 percent magnesium. Vitamin A was supplemented as a dry stabilized product.[4] When used, potassium bicarbonate was added to provide a level of 5 percent added potassium. The maximum ration fed was 4 pounds of the total mixture.

[4] Permadry, Stabilized Vitamins, Inc.

The basal diet was fed to all sheep for a period of 3 weeks before the experimental period. During this pre-experimental period free access was allowed to salt and bonemeal with the exception that salt with withheld from the group of four ewes which were to receive the low-salt, high-potassium diet. Because of its possible ameliorating effects, the bonemeal was withdrawn at the beginning of the experimental period.

Eight sheep fed no added potassium served as the control group. The remaining 22 ewes received the high potassium diet and were divided into five experimental lots. Two lots of five ewes each were given, respectively, free choice access to a crude sea magnesium oxide preparation, and to a crude calcium-magnesium sulfate mixture ("gyp cake") containing 6 percent magnesium. The latter mixture was consumed at an average rate of 15 gm. per animal per day. One lot of four ewes was supplied no magnesium supplement, another lot of four ewes no added salt, and the final lot of four ewes had access to both salt and magnesium supplement, but received a water supply prorated on the basis of the water consumption of the control animals. In all other groups water was supplied *ad libitum*. The animals were group-fed and were weighed every 28 days and at the termination of the experiment.

Blood samples were drawn after 12, 48 and 62 days. The serum from each animal was analyzed for magnesium, calcium, potassium, sodium, and total serum proteins. Calcium was determined by the method of Kramer and Tisdall (1921) as modified by Clark and Collip (1925); magnesium by a modification [5] of the method described by Kunkel, Pearson, and Schweigert (1947); potassium and sodium by flame photometer methods (Natelson, 1950) adapted to the Beckman flame photometer; and the serum protein by the colorimetric method of Weichselbaum (1946).

Results

Effects of Potassium Bicarbonate on Feed Intake and Weight Increase

The effects of the potassium bicarbonate on feed intake and gain in weight are summarized in table 1. Since no significant effects of magnesium supplementation were noted here, the first three high potassium groups have been averaged together. Following the introduction of the potassium bicarbonate into the diet, feed consumption

[5] Low viscosity polyvinyl alcohol (0.5 percent) was added to the hydroxylamine hydrochloride solution in order to stabilize more effectively the magnesium hydroxide-titan yellow lake in the magnesium determination.

decreased immediately, suggesting that decreased palatability was probably the primary factor involved. Refusal of feed was more apparent in the low salt group initially, but feed consumption by this group increased somewhat during the remainder of the experimental period. The group receiving the controlled water intake demonstrated the lowest average feed consumption. Observation of the sheep fed the potassium bicarbonate indicated marked individual fluctuation in day to day feed intake.

The decreased feed intake is reflected in the significantly lower weight gains in the potassium-fed sheep. The lowered rates of gain were more apparent when salt was not fed or when the water was limited.

TABLE 1. THE EFFECT OF HIGH LEVELS OF POTASSIUM ON FEED CONSUMPTION AND GAIN

Diet	No. of Animals	Average Daily Feed Intake		62-Day Weight Increase	
		First 7 Days	62 Days	Mean	Std. Dev.
		lb./sheep	lb./sheep	lb.	lb.
Basal	8	4.00	3.86	32.6	7.1
5% potassium	14	2.70	2.44	17.0**	10.5
5% potassium —low salt	4	1.93	2.49	10.7**	10.3
5% potassium —low water	4	2.59	2.19	11.8**	6.1

** Difference from control group significant at 1% level.

Effects on Serum Constituents

The serum magnesium levels are shown in table 2. It is to be noted that the magnesium content of serum was markedly lowered following the inclusion of potassium bicarbonate in the diet. The decrease in serum magnesium was highly significant statistically ($P<0.01$) by the end of 12 days on the high potassium diet and remained so throughout the remainder of the experimental period. Following the initial development of the hypomagnesemia, however, there was no further decrease in the level of serum magnesium. An unaccountable decrease in the serum magnesium was observed in the control animals at about the time milo gluten meal was used in place of the soybean oil meal in the diet, but the sheep receiving the potassium still showed significantly lower levels of serum magnesium.

TABLE 2. THE EFFECT OF DIETARY POTASSIUM BICARBONATE ON THE MAGNESIUM CONTENT OF BLOOD SERUM

(Mg. magnesium per 100 ml. serum)

Diet	No. of Animals	Days on Experiment							
		0		12		48		62	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Basal	8	2.59	0.25	2.53	0.29	2.02	0.32	2.65	0.21
5% K	4	2.62	0.27	1.67	0.11	1.90	0.14	1.85	0.28
5% K + Crude MgO	5	2.62	0.27	1.92	0.32	1.46	0.27	1.99	0.33
5% K + "Gyp cake"	5	2.86	0.40	1.56	0.32	1.68	0.09	1.87	0.16
5% K — Low NaCl	4	2.58	0.10	1.99	0.06	1.83	0.31	1.72	0.21[1]
5% K — Low water	4	2.68	0.30	1.75	0.45	1.65	0.12	1.74	0.15[1]
Av. high K	22	2.68	0.26	1.78	0.31	1.69	0.24	1.84	0.24

[1] One animal from group was sacrificed on 56th day to examine for possible kidney damage.

Ad libitum supplementation of preparations containing magnesium had little or no effect.

Aside from the magnesium level, there was no other marked alteration of the serum constituents (table 3). A hypocalcemia sometimes occurs in grass tetany (Russell, 1944), but no consistent decrease in serum calcium was noted here. In fact, at 62 days, the potassium-fed ewes showed significantly higher serum calcium levels ($P<0.01$) than did the control animals. An exception, however, appears in the group receiving the controlled water supply at 12 days. A comaprison of initial or pre-test serum calcium levels with the levels at the end of

TABLE 3. THE EFFECT OF DIETARY BICARBONATE ON SERUM CALCIUM, POTASSIUM, SODIUM AND PROTEIN

Category of Interest	Dietary Treatment							
	Basal Diet		5% K		5% K Low NaCl		5% K Low Water	
No. of anima's	8		14[1]		4[1]		4[1]	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Serum values for								
Calcium (mg./100 ml.)								
At 12 days	9.5	0.77	9.5	1.52	10.0	1.68	8.4*	0.50
At 62 days	10.2	1.77	11.1	0.85	12.6	0.98	13.7	0.64
Potassium (mg./100 ml.)								
At 12 days	18.8	3.9	22.1*	3.0	19.8	2.1	20.2	0.8
At 62 days	21.8	2.7	23.6	3.1	22.7	2.5	24.1	1.8
Sodium (mg./100 ml.)								
At 12 days	381	24	383	19	385	11	377	11
At 62 days	392	19	384	11	388	13	391	9
Protein (gm./100 ml.)								
At 12 days	6.64	0.54	6.94	0.41	6.98	0.46	7.00	0.73
At 62 days	7.23	1.26	7.57	1.00	7.57	0.95	7.30	1.80

[1] One animal removed at 56 days.
* Difference from control significant at 5% level.

12 days shows a decrease in this group which was statistically meaningful.

Although the serum potassium levels of the ewes fed the potassium bicarbonate were higher at the end of 12 days, the hyperkaliemia was not marked. No significant hyperkaliemia was noted after this period. The serum sodium and protein levels were essentially unaltered by the various dietary treatments.

Clinical Observations

Although a hypomagnesemia was produced by the feeding of large amounts of potassium bicarbonate, no tetany was observed. The lowest observed levels of magnesium, however, were no less than 1.0 mg. per 100 ml., and perhaps the hypomagnesemia was not severe enough to produce typical convulsions.

A mild, transient hyperirritability was apparent at 48 days in the group receiving no supplemental salt. These animals appeared to show increased excitability and marked polypnea upon slight exertion. A similar observation was noted also in a previous experiment by Colby and Burns (1951) following a reduction of supplemental salt fed to sheep receiving 4 percent potassium. The hypersensitivity was of short duration in both cases lasting for only about 7 days.

One ewe from each of three groups, the low water, low salt and magnesium oxide-supplemented groups, was sacrificed at 56 days and the kidneys examined histologically. No detectable pathological changes could be attributed to potassium toxicity.

Discussion

In view of the observations that the symptoms of grass tetany appear to be accompanied by a hypomagnesemia (Sjollema, 1932; Russell, 1944; Pearson *et al.*, 1949; Barrentine and Morrison, 1953), the results presented here suggest that a high potassium intake may be a contributory factor in grass tetany and wheat plant poisoning. Although no tetany was produced, it is to be noted that from a clinical standpoint the hypomagnesemia produced by feeding the potassium bicarbonate was comparatively mild, thus tetany should perhaps not be expected. It is significant, however, that the magnesium level of sheep serum can be lowered by the addition of potassium to a diet which by itself supports a normal level of serum magnesium.

One important item concerning these studies should be pointed out. Daily feed consumption was low and variable with the animals receiv-

ing the diets containing the potassium bicarbonate. The question thus arises that if the intakes of potassium were higher, would the hypomagnesemia become more pronounced. It is known that the appetite of sheep and cattle turned out to pasture on the young wheat plant is a voracious one, and hence the total daily intake of potassium is probably higher under such conditions than under the present experimental conditions. The data reported here suggest that a high potassium intake can under certain conditions alter the normal metabolism of magnesium and possibly that of calcium and sodium. The reason for the apparent lack of effect of high potassium diets on serum magnesium levels in the previous studies (Pearson *et al.*, 1949; Odell *et al.*, 1952; Eaton and Avampato, 1952) as contrasted to the present work remains obscure.

Summary

The inclusion of 5 percent of potassium as the bicarbonate in a cottonseed hulls—ground milo—soybean oil meal diet resulted in a significant lowering of the magnesium content of the serum. This hypomagnesemia was without clinical symptoms. The limitation of water or the deprivation of sodium chloride did not accentuate the hypomagnesemia.

The calcium, potassium, sodium and total protein content of the serum were not markedly altered by the ingestion of potassium bicarbonate. A mild hypocalcemia was evident early in the experimental period in sheep with the limited water supply, but after 62 days the potassium-fed sheep tended toward a hypercalcemia. A mild hyperkaliemia was evident at 12 days, but later disappeared. The sodium and protein contents of the serum were unaffected.

The inclusion of potassium bicarbonate in the diet decreased feed intake and gain. Deprivation of sodium chloride and water appeared to accentuate the decrease in rate of gain. A transient mild hypersensitivity was indicated in the animals receiving the low-salt, high-potassium diets.

The relations of these findings to the etiology of grass tetany is discussed.

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EXTRACELLULAR WATER AND DEHYDRATION IN SHEEP [1, 2]

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THE importance of individual inorganic elements in the nutritional physiology of the domestic ruminants has received increasing attention during recent years. This is especially true of sodium and potassium for ruminants (Dennis and Harbaugh, 1948; Pearson *et al.*, 1949; Curme *et al.*, 1949; Aines and Smith, 1952; and Daniel *et al.*, 1952) and for simple gastric species (Meyer *et al.*, 1950a, b). However, little or no attention has been given to the important relationship of these elements to the body fluids.

Extracellular water consists of the water of the blood plasma and the interstitial fluid including also the lymph as it is anatomically understood (Gamble, 1951). The interstitial fluid lying between the plasma and the tissues is in almost free communication with the plasma since all the diffusable constituents of the plasma pass readily through the capillary endothelium, and it is by virtue of this free communication that the plasma is enabled to maintain continuous interchange between different tissues. The great speed of interchange between the plasma and the extravascular fluid is indicated by the fact that, as measured with D_2O, 73 percent of the water (Flexner *et al.*, 1942) and 60 percent of the sodium (Merrell *et al.*, 1944) is exchanged with extravascular fluid every minute.

Physiologically considered, extracellular water is one of the two definite entities of the total body fluids. Studies of sodium-potassium antagonism and of the balance of sodium, potassium and chloride in sheep (Hix *et al.*, 1952) have led us to a great appreciation of the body fluids in general as criteria for direct measurement of the response

[1] A portion of a study supported by funds contributed by the Salt Manufacturer's Association, Detroit, Michigan.

[2] Contribution No. 193 from the Department of Animal Husbandry and No. 128 from the Department of Veterinary Medicine, Kansas Agricultural Experiment Station, Manhattan.

[3] A portion of a thesis presented as partial fulfillment of the requirements for the degree Doctor of Philosophy in Animal Physiology at Kansas State College.

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of sheep to sodium and potassium feeding and to sodium deprivation. The data herein reported were taken from two experiments conducted during the years 1950–51 (Expt. 1) and 1951–52 (Expt. 2). Only those data related to the extracellular water studies are reported, even though the experimental design is all-inclusive.

Experimental

The experimental lambs were either western-type black face wethers or of Columbia-Rambouillet breeding.

Experiment I, 1950–51

In a single feeding trial 50 lambs were divided unequally into four lots. All lots received a basal ration of corn and alfalfa hay for a feeding period of 96 days. In addition to the basal ration Lot 1 received 32.3 gm. of potassium bicarbonate, adjusting the Na:K ratio to 1:82. Lot 2 received 30.3 gm. of sodium bicarbonate, adjusting the Na:K ratio to 1:2. Lot 3 received salt *ad libitum* (average daily consumption, 21 gm.), thus by their own choice adjusting the Na:K ratio to 1:2. Lot 4 received the basal ration only, the Na:K ratio being 1:45.

After the feeding period, three wether lambs from each of the four lots were placed in metabolism stanchions for 21 days for determinations of mineral balance (Na, K, Cl) and feed digestibility. On the last day of the balance, blood samples were taken and analyzed for carbon dioxide (alkali reserve), non-protein-nitrogen, protein, hemoglobin and the elements calcium, phosphorous, magnesium, sodium, potassium, and chloride. On the same day, the Na:K ratios in Lots 2 and 4 were changed to 1:82 (formerly 1:2) and 1:2 (formerly 1:45), respectively, for the antagonism and sodium retention study. The antagonism and Na retention was studied for 7 days.

Experiment II, 1951–52

At the Garden City Branch Experiment Station, 120 lambs were divided equally into two lots. Both lots received a basal fattening ration of 1.26 pounds milo grain, 2.4 pounds Axtell sorghum stover, 0.2 pound soybean pellets, and ground limestone. In addition to the basal ration Lot 1 received salt *ad libitum*. After a feeding period of 94 days three wether lambs were selected from each of the two lots and brought to the Manhattan station for further study. The

regiment of salt versus no-salt was continued on a basal ration of 1 pound milo grain and 1 pound alfalfa hay. Lot 1 received 20 gm. NaCl per head daily. The lambs were placed in metabolism stanchions for an uninterrupted period of 29 days for determinations of mineral balance (Na, K, Cl), nitrogen balance, feed digestibility, and sodium-potassium antagonism. This procedure is indicated in table 1. Blood samples were taken as described in Experiment I. Sodium and potassium were determined in plasma, urine, feces, and feeds by flame photometry. The other constituents were determined by conventional chemical analysis. At the time the lambs were placed in the metabolism stanchions, their sheared body weights ranged between 81 and 91 pounds.

Extracellular Water Determinations

Extracellular water was determined simultaneously with total body water by procedures developed in this laboratory (Hix, 1952) for studies of the body fluids in ruminants as they are related to composition, using antipyrine (1-phenyl-2, 3-dimethylpyrazolone-5-one) for total body water as first suggested by Soberman *et al.* (1949) and sodium thiocyanate (NaSCN) for extracellular water. Equal volumes of 20 percent antipyrine and 20 percent NaSCN were mixed, giving a solution of 10 percent by volume of antipyrine and 10 percent NaSCN of which 10 ml. were injected intravenously into each lamb. Blood samples were taken 2 hours post-injection from the vein opposite to that injected and at hourly intervals thereafter for 3 hours. After diphasic separation of antipyrine and $^{-}$SCN from serum, quantitation of antipyrine was determined with the Beckman spectrophotometer in the ultraviolet at λ=350 mu and $^{-}$SCN with the Coleman model 14 at λ=490 mu.

By plotting the diminishing densities of antipyrine and $^{-}$SCN in the serum samples against time and extrapolating to zero time (t_0), the serum concentration of antipyrine and $^{-}$SCN at the time of injection was determined. The dilution of these substances in the body fluids was taken as a measure of the total body water and extracellular water respectively.

Results and Discussion

The extracellular fluids fill a number of subcompartments, each occupied by a continuous fluid phase enclosed within a more or less

continuous membrane. They include the plasma water and a number of other collections of fluid. The total extracellular space has been measured by the dilution volume of thiocyanate ($^-$SCN) which passes freely through the vascular wall and equilibrates throughout these subcompartments.

In accordance with considerations stated elsewhere (Elkinton, 1950), the volume distribution of $^-$SCN apparently measures a fluid volume intermediate between the true extracellular water (ECW) volume and the total body water (TBW) in the dog and man. Consequently, no evidence can be presented as to the exact relation of the $^-$SCN space to the true ECW volume in sheep. The $^-$SCN space, as determined, is consistently reproducible in any given individual and changes in the $^-$SCN space may reasonably be taken to measure changes in the true ECW volume. For this purpose it is considered a valid and useful experimental technique.

Volume of Extracellular Fluid

The extracellular fluid volume can be determined by a number of substances which diffuse freely from the vascular compartment but do not appreciably enter the body cells or intracellular fluid phase. Most notable among the many available substances is inulin (Guadino *et al.*, 1948) which shows a volume distribution of 21–33 percent of body weight in dogs and 15–16 percent in man. Because of the need for a constant intravenous infusion of inulin, after a priming dose, it is not adaptable to field conditions. The $^-$SCN space in dogs varies between 33 and 35 percent (Elkinton and Taffel, 1942), and simultaneously determined Na^{24} and $^-$SCN spaces in 39 rabbits average 26 and 22 percent respectively (Aikawa, 1950).

Aside from this ECW there is water in special compartments of the body, such as cerebrospinal fluid, water in the bile, synovial sacs, bladder, and water in the lumen of the glands and the gastrointestinal tract. None of this water is intracellular, yet in reaching these sites it has to pass through cells or be processed by cells. Edelman *et al.* (1952a) have termed this "transcellular water" and conclude that in man "the amount of water outside of body cells appears to be between 25 and 30 percent of body weight." Since $^-$SCN may be assumed to pass into the saliva (Wood and Williams, 1949), it is further assumed that the $^-$SCN space includes a large part of this transcellular water.

Many simultaneous determinations of ECW and TBW on goats, sheep, and cattle, normally hydrated, reveal that the ECW is extremely

consistent in volume at 30 percent of body weight. Among these ruminants there exists a species difference in the metabolism of ^{-}SCN (unpublished data).

In table 1 the means by which the animals were hydrated and dehydrated are indicated. The ECW volume observed at the end of the mineral balance period must be respected as indicating the influence of salt supplementation and of salt deprivation. It appears, even though the data are limited, that the deprivation of supplemental salt in lambs on a practical fattening ration results in dehydration of the body fluids. More obvious, however, is the difference in degree of dehydration by feeding $KHCO_3$ to lambs with an apparent store of Na (lambs 26 and 56) and to one with little or no reserve Na (lamb 194). In a previous experiment (Hix *et al.*, 1952), the feeding of $KHCO_3$ to lambs during the fattening period (96 days) dehydrated their body fluids by approximately 4 percent of body weight, equivalent to a loss of 1.7 liters of water per animal. The administration of NaCl to one lamb (No. 220), deprived of supplemental salt for approximately 7 months, hydrated the animal to an increase in ECW of about 14 percent. The feeding of silage brings about a loss of extracellular Na and consequently a reduction in ECW volume through Na diuresis. A water diuresis in dairy cattle has been observed to produce a similar effect (Sellers and Roepke, 1951). The differences in degree of dehydration in lambs 19 and 228 is attributed to differences in the amount of silage consumed, even though the influence of an exchangeable Na store cannot be ruled out.

As additional evidence figure 1 illustrates the effectiveness of $KHCO_3$ in producing a Na diuresis which varies both in extent and duration among individual lambs similarly treated.

The quantitative extent of the negative Na balance for the 7-day period (fig. 1) is quite similar for the three lambs (table 2); however, the initial response to $KHCO_3$ varies widely as evidenced by the excretion of 874 percent of the dietary Na intake within 12 hours by lamb 99, 592 percent by lamb 71, and 267 percent by lamb 56. With the exception of a single 12-hour period, lamb 56 was in negative Na balance throughout the entire seven days.

Calculation of Changes in Extracellular Fluid Volume from Sodium Balance

Sodium exists in a freely diffusable state in the plasma water as shown by the fact that its distribution between plasma and pleural, peritoneal, and subcutaneous fluids (all extracellular) is consonant

TABLE 1. EXPERIMENTAL TREATMENT AND CHANGES IN EXTRACELLULAR WATER (ECW)[1]

Lot and Animal No.		End of Mineral Balance 4–21–52 ECW Liters	End of Mineral Balance 4–21–52 ECW Percent Body Weight [4]	12 Days Experimental Treatment	End of Na:K Antagonism 5–3–52 ECW Liters	End of Na:K Antagonism 5–3–52 ECW Percent Body Weight	End of Na:K Antagonism 5–3–52 Percent Change in ECW		68 Days 7–10–52 ECW Percent Body Weight	58 Days 9–6–52 ECW Percent Body Weight
I	19 [2]	13.021	32.1	2 lb. silage	11.807	28.6	—9.3	....	....	....
	26	12.886	31.8	20 gm. $KHCO_3$	12.564	30.8	—2.5	....	....	....
	56	13.441	31.8	20 gm. $KHCO_3$	12.895	30.3	—4.1	....	....	....
	Group ave.	13.116	31.9							
2	194 [3]	10.435	28.5	20 gm. $KHCO_3$	9.159	25.3	—12.2	Basal	25.9	24.9
	220	11.161	29.2	20 gm. NaCl	12.703	33.2	13.8	....	....	....
	228	11.940	28.8	2 lb. silage	11.530	28.5	—3.4	Basal	26.7	24.7
	Group ave.	11.179	28.8							

[1] Data from Experiment II, 1951–52.
[2] During mineral balance study the quantitative Na, K, Cl intakes were 8.151 gm., 9.670 gm., and 13.058 gm.
[3] Basal unsupplemented group. The Na, K, Cl intakes were 0.211 gm., 9.670 gm., and 0.998 gm. during balance study.
[4] All lambs were closely sheared of wool after each ECW determination and the percent ECW is based on the wool-free weight.

with the Gibbs-Donnan equilibrium (Folk *et al.*, 1948), and as mentioned previously, the exchange between these fluids is rapid. Changes in the quantitation of body Na will therefore bring about a change in ECW volume in the endeavor to maintain its iso-osmolarity. These changes can be estimated with reasonable accuracy from the balance of Na and changes in serum Na concentration by the formulation of Peters (1944), modified as follows for greater clarity.[5]

$$ECW = \frac{ECW_1 \times \left(\frac{Na_s}{W_s} \times 0.95 \right) \pm Na_{bal.}}{\frac{Na_{s_2}}{W_s} \times 0.95}$$

where:

ECW_1 = Initial ECW volume (calculated)
Na_s = mEq. Na in serum; initial
Na_{s_2} = mEq. Na in serum; final
W_s = serum water (100 − gm. % protein/100)
$Na_{bal.}$ = mEq. Na balance; positive or negative
0.95 = Donnan correction factor for Na

By the equation it is required that some value for ECW_1 be assumed; however, since by direct determination of ECW it has been observed that lambs receiving NaCl or $NaHCO_3$ show 32 percent ECW and those deprived of salt show 29 percent ECW, these figures were used to calculate ECW_1 in the equation from body weight. The balance of Na and calculated ECW changes for six lambs from Experiment 1, 1950–51 are indicated in table 2.

The calculated response of lambs to $KHCO_3$ administration (previously receiving $NaHCO_3$) is in agreement with the data of table 1 in which ECW was determined directly. However, the degree of hydration due to NaCl administration to Na deficient lambs is high, even though not unusually so. Wesson *et al.* (1950) expanded the ECW volume in six dogs 25–50 percent above normal, which held constant for 8 hours. A dilution of plasma protein concentration occurred. No change in plasma protein or the principal electrolytes in states of hydration in lambs has been observed, and in view of the recent study of Edelman *et al.* (1952b), in which it was demon-

[5] Since in all determinations there were no significant serum Na changes and all serum protein concentrations were very near 6.5 gm. percent, the relation $Na_s/W_s \times 0.95$ becomes constant at 154 mEq. Na in the interstitial fluid. This constant was used in all ECW calculations and the above equation simplifies to $ECW = (ECW_1 \times 154) \pm Na_{bal.}/154$.

strated that the normal storage of Na in the bones of dog and man constitutes 35 percent of the total body Na. it is assumed that a considerable amount of the retained Na is stored and thus does not contribute in its entirety to expansion of the ECW volume.

Whereas the degree of dehydration as calculated may be considered permanent and static, the excessive hydration resulting from administration of NaCl is apparently transitory. The sodium retention

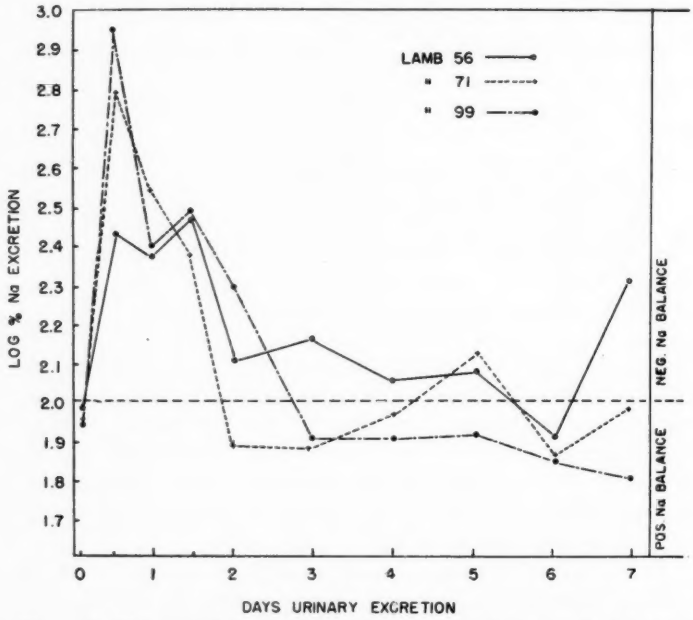

Figure 1. The excretion of sodium via the urine by three lambs for 7 days during the administration of 32.3 gm. of $KHCO_3$ per head daily. The lambs had previously received 30.3 gm. of $NaHCO_3$ per head daily for 117 days.

studies (Lot 4, table 2) reveal that after administering 21 gm. of NaCl (equal to 8.34 gm. Na) daily for 7 days to lambs deficient in Na, only 37 percent of the Na intake is excreted within 12 hours, 39 percent within 24 hours, and after 36 hours 112 percent is excreted. Between the second and seventh days there is a steadily decreasing retention of Na. This general trend to exceptionally high Na retention followed by a negative Na balance 36–48 hours after continuous

administration of NaCl has been observed repeatedly. Consequently, there is a rapid expansion of ECW in the early Na retention stage due to rapid transference of intracellular water to the ECW compartment (Crawford and Guadino, 1952) which is followed by an abrupt check in its expansion as demonstrated by a negative Na balance. The renal mechanism is induced to an increased excretion of Na in the endeavor to establish a normal balance in the body fluids. So long as the NaCl administration continues, the sheep's body remains mildly hydrated as demonstrated in table 1. This aspect of the study is receiving more attention as it offers a possible explanation to the etiology of NaCl intoxication, not uncommon in feeder lambs. It is tempting, on this and other evidence (Holmes and Gregersen, 1950;

TABLE 2. CALCULATED CHANGES IN ECW FROM Na BALANCE[1]

Lot and Animal No.	Weight Kg.	Initial ECW Liters	Gm. Na Balance 7-Days	Final ECW Liters	Volume Change Liters	Percent Change in ECW
2[2] 56	46.12	14.76	−1.373	14.37	−0.39	−2.6
71	47.28	15.13	−1.286	14.76	−0.37	−2.4
99	52.15	16.69	−1.619	16.23	−0.46	−2.8
4[3] 31	45.21	13.11	16.252	17.69	4.58	34.9
69	48.67	14.11	3.888	15.21	1.10	7.8
74	48.77	14.14	16.087	18.67	4.53	32.0

[1] Data from Experiment I, 1950–51; Na:K antagonism and Na retention study .
[2] Lot 2 receiving 32.3 gm. $KHCO_3$ per head daily. Formerly received 30.3 gm. of $NaHCO_3$.
[3] Lot 4 receiving 21.0 gm. NaCl per head daily. Formerly received the basal unsupplemented ration.

Pistor *et al.*, 1950), to suggest that NaCl intoxication or "salt poisoning" is enhanced when the Na-deficient and dehydrated lambs gain access to salt. Already dehydrated, the ingested salt may result in further cellular dehydration and rapid expansion of ECW volume, creating a great thirst drive which could not be immediately satisfied because of the loss of all sense of physiologic balance.

Quantity of Sodium in the Body

From analyses of edema fluids and by direct sampling of ECW from muscles, it has been established that the interstitial fluid has the same chemical anatomy as an ultra-filtrate of plasma, differing from plasma only in containing smaller quantities of protein (Gamble, 1951). The cations in this interstitial fluid consist chiefly of Na^+, while the principal anions are Cl^- and $^-HCO_3$. The total quantity of

Na in the body could be accounted for if it were confined to a volume equal to the ^{-}SCN space or ECW volume, and if this ECW were quantitatively homogenous with respect to Na; however, the homogeniety of the ECW is questionable since 35 percent of the total body Na of man and the dog resides in bone, and of this amount 55 percent is bound chemically in bone matrix (Edelman *et al.*, 1952b). The ECW does not contain all body Na, but by making use of this disposition of body Na in a calculation of total body Na, it is possible to approximate the total amount of Na in the body from determinations of serum Na and ECW volume. Furthermore, differentiation of physiologically active or exchangeable Na and chemically bound Na is possible.

The following equations have been derived for the estimation of extracellular Na (EC_{Na}), total body Na (TB_{Na}) and total exchangeable Na ($TExch_{Na}$), or that quantity capable of physiologic activity and thus contributing to maintenance of the normal ECW volume.[6]

$$(1) \qquad EC_{Na}=ECW\times\left(\frac{S_{Na}}{S_w}\times 0.95\right)$$

$$(2) \qquad TB_{Na}=EC_{Na}+\frac{EC_{Na}}{1.857}$$

$$(3) \qquad TExch_{Na}=EC_{Na}+\left[\frac{EC_{Na}}{1.857}-\left(\frac{EC_{Na}}{1.857}\times .055\right)\right]$$

The validity of the equations must rest on the premise that: (a) the ^{-}SCN space is a measure of the true ECW volume, (b) 35 percent of the total body Na resides in bone, (c) 55 percent of the bone Na is chemically bound and not exchangeable and (d) sufficient dietary Na must be available in order to provide a normal disposition of body Na as would be exemplified in a normal EC_{Na}: $bone_{Na}$ ratio. As the exchangeable bone Na doubtless serves as a Na store in times of plenty, it is assumed to be available for maintenance of the ECW volume in metabolic emergencies. Sodium-deprived lambs

[6] Derivation of equation (1) is as follows. The Na^+ of serum (S_{Na}) was 354 mg. percent, the protein 6.5 gm. percent—therefore, 354/0.935=379 mg. percent Na^+ in serum water. The N^+ of interstitial fluid=379×0.95=360 mg. percent. The volume of the extracellular water in liters (ECW)×(360×10)/1000=gm. Na^+ in the extracellular entity. The factor 0.95 is used to correct for the Gibbs-Donnan effect. In equation (2) the factor 1.857 was derived from the study of Edelman *et al.* as follows: If 35 percent of the total body Na resides in bone, then the EC_{Na} as determined represents 65 percent of the total, and the ratio of EC_{Na} to TB_{Na}=65/35=1.857 and EC_{Na}/1.857=total Na of bone. The factor 0.55 in equation (3) accounts for that amount of the total Na of bone that is bound (55 percent) and is thus not exchangeable. The total Na of bone (EC_{Na}/1.857) minus that chemically bound is equal to exchangeable bone Na^+, and exchangeable bone Na^+ plus EC_{Na} comprises the total exchangeable Na^+ of the body.

may therefore be expected to have utilized this store to exhaustion, at which time dehydration becomes established. As indicated in table 1, lambs 26 and 56, possessed of Na stores, show a decrease of only 2.5–4.1 percent in ECW after 12 days of $KHCO_3$ administration. Receiving the same treatment but lacking an equal store of Na, lamb 194 shows a decrease of 12 percent in ECW after 12 days. The loss of EC_{Na} apparently could not be replaced because of a deficiency of exchangeable bone Na.

Total body Na estimations, by the above equations, for lambs receiving adequate dietary Na (Nos. 19, 26 and 56) and with an average sheared body weight of 41.10 kg. reveal the following.

Extracellular Na (EC_{Na})=47.23 gm.
Total body Na (TB_{Na})=72.66 gm.
Total exchangeable Na ($TExch_{Na}$)=58.67 gm.

The EC_{Na} of Na-deficient lambs (194, 220 and 228) show an average of 1.04 gm./kg. body weight as compared to 1.15 gm./kg. for the Na-supplemented lambs. Since no serum Na changes were observed, these values obviously are due entirely to differences in ECW volume and thus the degree of hydration. A TB_{Na} content of 72.66 gm. is equal to 0.177 percent body weight. This figure agrees with 0.182 percent for lambs (Evvard *et al.*, 1926), 0.192 for calves (Hogan and Neirman; cited by Dukes, 1947) and 0.15 percent for man (Sherman; cited by Brody, 1945), all on a fresh weight basis. Recalculation of the data of Harrison *et al.* (1936) reveals the following values for total body Na as percent of body weight: rabbit, 0.135; dog, 0.161; and monkey, 0.147–0.182.

A $TExch_{Na}$ of 58.67 gm. is equivalent to 1.42 gm. or 61.7 mEq. Na/kg. body weight. Exchangeable Na determinations in 11 men, using Na^{24} (Edelman *et al.*, 1952a), average 41.4 mEq./kg. body weight with a range of 36–46 mEq. The range is equivalent to 0.83–1.06 gm. Na/kg. The value for sheep, as calculated, is slightly higher than that of man, but this is to be expected since the ruminant species apparently contain more TB_{Na} than man, and the ECW volume as determined with ^-SCN can only be considered to measure a definite fluide volume which approximates the true ECW volume. There are also certain anatomical features of the ruminants which favor a greater ECW volume on a unit weight basis when compared to man and the dog.

Lambs 194 and 228, after completion of the Na:K antagonism study as indicated in table 1, were placed on a basal ration of grain

and alfalfa without supplemental salt. After 138 days from the time the first ECW determination was made, their ECW volume decreased by 1.73 and 1.86 liters, representing 6.23 and 6.69 gm. of extracellular Na and thus dehydration of ECW by 12.6 and 14.0 percent, respectively.,

As estimated for Na-supplemented lambs, $TExch_{Na}=58.67$ gm., $EC_{Na}=47.23$ gm., and $58.67-47.23=11.44$ gm. of exchangeable bone Na_+, which in metabolic emergencies could contribute to maintenance of the ECW volume. With this amount of exchangeable bone Na_+, approximately 390 days of supplemental salt deprivation would be required to dehydrate these lamb's ECW to a level comparable to that of lambs 194 and 228. Lambs 194 and 228 had no supplemental salt for 339 days. The estimation of exchangeable bone Na_+, therefore, lends credence to the method of calculation. The wether lamb obviously has a particular faculty for conserving bodily Na. During the mineral balance study (Expt. II, 1951–52), the three lambs deprived of supplemental salt excreted an average urine volume of 115, 239 and 180 ml. daily, and one lamb excreted as little as 40 ml. in a single 24-hour period. This urine was of a deep brown-colored syrupy consistency and its specific gravity was such that it could not be determined with a urinometer. Simultaneously determined renal clearances of creatinine, Na, K, and Cl on these lambs revealed an insignificant reduction in renal glomerular filtration rate, and of the quantity of Cl filtered by the glomeruli, 99.91 percent was later reabsorbed by the renal tubules, leaving only 0.09 percent to be excreted. This ability to conserve Na and Cl resides principally in the ability of the tubules to absorb almost completely all Na and Cl filtered from the ECW by the glomeruli. Under such physiologic control severe dehydration of the extracellular fluid in this species would require a considerable length of time, conditioned, however, by the exchangeable bone Na reserve and the antagonistic action of K.

Hydration Versus Dehydration

In the feedlot test (Expt. II, 1951–52) salt-supplemented lambs, of which lambs 19, 26, and 56 are representative, gained 0.296 pound daily (average of 60 lambs) while the lambs on the basal unsupplemented ration, of which lambs 194, 220, and 228 are representative, gained 0.262 pound daily (average of 60 lambs) (Bell and Erhart, 1952). Even though the lambs indicated in table 1 were selected from the above lots for uniformity in weight and finish, their degree of

hydration may be taken as representative of the salt-supplemented lot. As 3 percent more of their body weight consists of retained water, as compared to the unsupplemented lambs, the daily gain minus the retained water becomes 0.262, identical to the gain shown by 60 lambs on the same ration but without supplemental salt. The product produced is therefore no greater when compared on an equal basis of hydration, but due to differences in feed requirement per cwt. of gain, salt-supplemented lambs were more efficient gainers. Mild dehydration is obviously not conducive to the maximum efficiency complex when produced by salt deprivation.

Summary

The volume of the extracellular fluids in sheep normally hydrated, as measured by the dilution of thiocyanate (^{-}SCN), is equal to 30 percent of the sheared body weight. The extracellular fluid volume is extremely consistent in any given individual.

Sodium chloride and sodium bicarbonate are hydrating while potassium bicarbonate is dehydrating to lambs. Dehydration due to high dietary potassium intake is produced by a resulting sodium diuresis which may be continuous or transitory.

Administration of sodium chloride to lambs deficient in sodium expands the extracellular fluid volume rapidly and provides for the storage of sodium, much of which is exchangeable and may, in metabolic emergencies, contribute to maintenance of the normal extracellular fluid volume.

Equations are presented for estimation of total body, total exchangeable, and extracellular sodium from determinations of serum sodium and extracellular fluid volume.

Sodium chloride supplementation to lambs in the amount of 20 gm. daily hydrates the extracellular fluids to an increase of 3 percent of body weight as compared with unsupplemented lambs. The superior gains of salt-supplemented lambs appear to be due to retention of water as a result of hydration.

The sheep has a particular faculty for conserving body sodium. With two lambs, after 339 days of supplemental salt deprivation, the extracellular fluid volume decreased 13–14 percent of the normal value.

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A STUDY OF ELEMENTAL SULFUR AND OF SOME OTHER SUGGESTED PREVENTIVES OF ENTEROTOXEMIA IN A LAMB FATTENING RATION [1]

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SEVERAL methods have been proposed during the last several years to reduce losses in fattening lambs due to enterotoxemia. Christensen *et al.* (1947) and Deem *et al.* (1948) demonstrated that elemental sulfur added to a fattening ration for lambs reduced death losses from enterotoxemia. They also reported lowered feed consumption and lowered rate of gain which resulted in increased cost of gain. The increased cost of gain, however, was more than offset by reduced death losses so that the practice of adding one-third to one-fourth ounce of sulfur per head per day to a fattening ration for lambs was advocated.

Vaccination of feeder lambs with *Clostridium perfringens* bacterin to reduce death losses due to enterotoxemia (Whitlock and Fabricant, 1947) is now a common practice. Some feeders believe that such vaccination of feeder lambs reduces their subsequent gains. This belief has resulted in reduced use of vaccination in some lamb-feeding areas.

Cox *et al.* (1948; 1949) reported varying results from the use of sodium bicarbonate to reduce digestive disturbances in lambs on a fattening ration.

Feeders generally use a concentrate:roughage ratio of approximately 1:1. It is a common practice of feeders to reduce the concentrate fed when difficulty is experienced with enterotoxemia; this in turn results in reduced rate of gain.

Two experiments with feeder lambs were planned to study some of the effects of the various treatments suggested for control of enterotoxemia. Since numerous experiments by Colorado workers have shown that the addition of elemental sulfur to a lamb fattening ration results in lower death losses from enterotoxemia, lower feed consumption and lower rate of gain, these experiments were designed primarily

[1] This is the first paper in a series on sulfur in sheep nutrition.

[2] Acknowledgment is made for the assistance of W. J. Hampton, Sheep Farm Foreman, and his associates.

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to test: (1) sulfur as a specific prophylactic agent for preventing death from enterotoxemia in the presence of conditions that might otherwise cause such a death; (2) the efficiency of feed utilization of lambs as judged by gain in weight when on equal intakes of feed supplemented with the preventives.

Experimental Procedure—First Experiment

Three lots each of 45 black-faced, western feeder lambs were used in this experiment. All lambs were dipped in an arsenical dip and treated with phenothiazine prior to starting them on experiment. Individual lamb weights were taken at the beginning and at 28-day intervals during the experimental period of 84 days (Nov. 17 to Feb. 9).

Lot 1 and 2 lambs were hand-fed equal feed by lots twice daily. Lot 3 lambs were hand-fed relatively less roughage and more concentrate than the lambs in lots 1 and 2. All lambs were started on a daily feed of 4 pounds of corn silage and 0.25 pound of ground corn supplemented with 0.25 pound per head per day of a protein-calcium supplement. Corn for lots 1 and 2 was increased at the rate of 0.25 pound per head per day each week until the lambs in lots 1 and 2 were eating approximately 1.25 pounds of corn per day and 2.5 pounds of silage. At that time (sixth week) the lambs in lot 3 were receiving 1.6 pounds of corn and 2 pounds of silage.

The corn fed was ground so that elemental sulfur could be thoroughly mixed with that fed the lambs in lots 2 and 3. The sulfur was added at the rate of 2 percent by weight of the corn for the first 5 weeks of the trial. During the remaining 7 weeks of the trial, the level of sulfur was reduced to 1 percent in order to maintain reasonable feed consumption.

All lambs which died during the experiment were autopsied and the cause of death determined, where possible.

Results and Discussion of First Experiment

Differences between lots in average daily gains were not significant (table 1). The lambs in lot 2, receiving a supplement of sulfur, gained as well as similar lambs fed the same amounts of similar feeds without sulfur. Under these conditions sulfur did not appear to reduce the efficiency of feed utilization. There were no death losses in lot 2 (sulfur) while there were three in lot 1 (no sulfur). One lamb in lot 3 receiving the high concentrate-low roughage ration with a sulfur

supplement died. An autopsy, conducted by the Department of Veterinary Pathology and Hygiene of the University of Illinois Veterinary College, indicated that the probable cause of death was enterotoxemia. Sulfur, in this case, apparently did not act as a specific prophylactic agent in preventing enterotoxemia where the proportion of concentrate to roughage was made high intentionally. Weight changes and feed consumption figures are given in table 1.

TABLE 1 SULFUR IN RATIONS FOR FATTENING LAMBS IN DRY LOT—SUMMARY OF DATA FOR FIRST EXPERIMENT

(pounds)

	Lot 1 No Sulfur	Lot 2 Sulfur	Lot 3 High Corn and Sulfur
No. of lambs completing experiment	42[1]	45	44[2]
Average final weight [3]	96.7	97.6	97.5
Average initial weight [3]	74.1	74.4	74.3
Average daily gain [3]	0.27	0.28	0.28
Average feed daily			
Corn silage	3.0	3.0	2.7
Ground corn	1.1	1.1	1.3
Soybean oil meal and mineral [4]	0.26	0.26	0.26
Feed per hundredweight gain [5]			
Corn silage	1125	1095	961
Ground corn	395	389	462
Soybean oil meal and mineral	97	95	93

[1] Lamb 1995 died December 5, 1947, positive test for enterotoxemia; lamb 1945 died December 24, 1947, and lamb 1946 died January 9, 1948. Autopsies indicated the probable cause of death was enterotoxemia in each case.

[2] Lamb 1969 died December 23, 1947. Autopsy indicated probable enterotoxemia.

[3] Only weights and gains of lambs which completed the trial are used in this table.

[4] Supplement consisted of 80 percent soybean oil meal, 10 percent iodized salt and 10 percent powdered limestone.

[5] Prorated feed deducted on the basis of lamb-days for the lambs which died.

Experimental Procedure—Second Experiment

Four lots, each consisting of 50 black-faced, western feeder lambs, were used in a second experiment similar to the first experiment. All lambs were dipped in an arsenical dip and treated with phenothiazine prior to starting them on feed. Individual lamb weights were taken at the beginning and at 28-day intervals during the experimental period of 84 days (Oct. 18 to Jan. 9). Half the lambs in each lot selected at random were vaccinated during the first week of the feeding period with 5 cc. of *Clostridium perfringens* Type D bacterin.

All lots of lambs were hand-fed equal feed twice daily. The lambs

were started on a feed of 4 pounds of corn silage and 0.25 pound of corn-and-cob meal supplemented with 0.25 pound of a mixture of a protein-calcium supplement. Corn-and-cob meal was increased each week at the rate of 0.25 pound per head per day until the lambs were eating about 1.5 pounds daily. The lambs were eating 1.6 pounds per head per day at the twelfth week. Lot 1 lambs served as controls. Lot 2 lambs received a supplement of approximately one-third ounce of powdered sulfur per lamb per day dusted over their feed. Lot 3 lambs received a supplement of approximately one-ninth ounce of bicarbonate of soda per lamb per day dusted over their feed. Lot 4 lambs received a supplement of corncobs equivalent to doubling the cob in corn-and-cob meal. This was done by adding 20 pounds of cob to each 80 pounds of corn-and-cob meal. Lot 4 lambs received this extra cob in addition to feed equivalent to that fed lots 1, 2, and 3.

During the last 3 weeks of the experiment the corn silage for lot 4 was reduced half in order to force higher consumption of corn-and-double-cob meal.

Results and Discussion of Second Experiment

Lambs in lot 2 gained significantly more ($P<0.05$) than those in lot 1. Differences in gains in weight between all other lots were not significant.

The lambs vaccinated with 5 cc. of *Clostridium perfringens* Type D bacterin gained 0.310 pound per head per day as compared with 0.308 pound per head per day for the lambs which were not vaccinated.

It is probable that faulty vaccination technique, rather than any difficulty from the bacterin itself, has lead to the belief that vaccination of lambs reduces their subsequent gains. Packers have reported that abscesses have been trimmed from numerous lamb carcasses, presumably from infections caused at time of vaccination. Such infections from faulty vaccinations could reduce gains.

Weight changes and feed consumption figures are given in table 2.

Summary and Conclusions

Two experiments were conducted using black-faced, western feeder lambs group fed common Corn Belt fattening rations varied by additions of elemental sulfur, bicarbonate of soda or extra corncobs, by increased concentrate:roughage ratio and by vaccination. The following conclusions are drawn:

1. Elemental sulfur added to a ration of corn silage, corn, soybean

oil meal, and powdered limestone did not reduce efficiency of feed utilization as judged by gain in weight when equal feed was fed, and apparently did not completely eliminate losses from enterotoxemia.

2. Elemental sulfur added to a ration of corn silage, corn-and-cob meal, soybean oil meal, and powdered limestone increased the efficiency of feed utilization as judged by gain in weight when equal feed was fed.

TABLE 2. SULFUR, BICARBONATE OF SODA AND EXTRA CORNCOB IN RATIONS FOR FATTENING LAMBS IN DRY LOT—SUMMARY OF DATA FOR SECOND EXPERIMENT

(pounds)

	Lot 1	Lot 2	Lot 3	Lot 4
No. of lambs	50	50	50	50
Average final weight	101.5	105.2	103.0	102.5
Average initial weight	77.0	77.1	76.9	77.2
Average daily gain	0.29	0.33	0.31	0.30
Average feed daily				
Corn silage	3.7	3.7	3.7	3.3
Corn-and-cob meal	1.2	1.2	1.2	1.1
Soybean oil meal and mineral [1]	0.27	0.27	0.27	0.27
Sulfur	....	0.02	....	....
Bicarbonate of soda	....	....	0.007	....
Ground cobs	....	....	....	0.2
Feed per hundredweight gain				
Corn silage	1255	1095	1182	1104
Corn-and-cob meal	393	343	370	368
Soybean oil meal and mineral	94	82	88	91
Sulfur	....	6.83	....	....
Bicarbonate of soda	....	....	2.44	....
Ground cobs	....	....	....	74

[1] Supplement consisted of 80 percent soybean oil meal, 10 percent iodized salt, and 10 percent powdered limestone.

3. Lambs vaccinated with 5 cc. of *Clostridium perfringens* Type D bacterin to prevent enterotoxemia made as good gains in weight as did lot mates not vaccinated.

4. No significant effect on gain was obtained from the addition of sodium bicarbonate or extra cob to a ration of corn silage, corn-and-cob meal, soybean oil meal, limestone, and iodized salt.

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THE UTILIZATION OF FEED NITROGEN BY LAMBS AS AFFECTED BY ELEMENTAL SULFUR [1, 2, 3, 4]

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UREA offers an alternative source of nitrogen for ruminant feeding. During World War II it came into prominence in America due to the shortage of commonly used protein supplements. The utilization of urea is thoroughly discussed in a recent review by McNaught and Smith (1947). Hamilton *et al.* (1948) found that urea is as satisfactory a source of nitrogen for growing lambs as are most ordinary protein supplements, provided at least 25 percent of the feed nitrogen is in the form of preformed protein and provided further that the total protein equivalent does not exceed 12 percent.

There seems to be agreement that the non-ruminant is unable to utilize inorganic sulfur (Daniels and Rich, 1918; Lewis and Lewis, 1927; Kellerman, 1933; Tarver and Schmidt, 1939; Evvard *et al.*, 1925), but there are differences of opinion in regard to its utilization by the ruminant.

DuToit *et al.* (1936) failed to confirm the finding of Steyn (1931) that elemental sulfur increased the growth rate and wool yield of lambs. Garrigus *et al.* (1950) trio-fed growing-fattening lambs (1) a methionine-deficient basal ration, (2) the basal ration supplemented with elemental sulfur and (3) the basal ration supplemented with methionine. They found that the basal ration was improved significantly by the addition of methionine and that differences between the lambs fed the basal ration and the sulfur-supplemented ration approached significance. Hale and Garrigus (1953) fed one lamb radioactive sulfur and another radioactive sulfate. Within 2 weeks radioactive sulfur was detected in the cystine of the wool protein of

[1] This paper is a part of a thesis submitted by the senior author to the Graduate College, University of Illinois, in partial fulfillment of the requirements for the degree of the Doctor of Philosophy in Animal Science.

[2] Published with the approval of the Illinois Agricultural Experiment Station.

[3] This is the fourth paper in a series on sulfur in sheep nutrition.

[4] We wish to express acknowledgment with thanks to Dr. P. D. Beamer and Associates of the College of Veterinary Medicine, for conducting autopsies, and to Dr. M. F. James of the Animal Science Department for her excellent cooperation in the histological study.

Alpha tocopherol, thiamine hydrochloride and riboflavin were generously supplied by Merck and Company through the courtesy of Dr. Randolph T. Major.

E. I. duPont de Nemours and Company, Wilmington, Delaware, generously provided urea through the courtesy of Dr. M. F. Gribbins.

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each lamb. Block and Stekol (1950) found that the cystine and methionine in the milk proteins of a cow showed appreciable radioactivity several days after radioactive sodium sulfate was fed. Thomas *et al.* (1951) obtained nitrogen and sulfur balances on lambs fed a purified ration with and without sulfates. The lambs on sulfates lived while the basal lambs died. Since their trial was conducted by using *ad libitum* feeding and the basal lambs had a low feed intake while the sulfate-supplemented ones had a relatively high feed intake, it is difficult to determine what effect the sodium sulfate actually had upon the retention of dietary nitrogen.

In the present experiment nitrogen and sulfur balance trials were conducted to determine the influence of elemental sulfur on nitrogen retention by lambs fed a purified ration, low in sulfur, and containing urea as the chief source of nitrogen, and to determine the utilization of elemental sulfur. Responses to supplementation with elemental sulfur in relation to body growth, wool growth, and wool composition were also studied.

Experimental

Four grade Suffolk and four grade Shropshire wether lambs, weighing 37 to 52 pounds, were paired according to age, weight, breed, and general appearance, and fed experimentally over a period of approximately 90 days. The first member in each pair was fed the basal ration (Ration A), a modification of the Thomas *et al.* (1951) ration, shown in table 1. The second member was fed the basal ration plus 3 grams of elemental sulfur per pound of ration (Ration B). According to analysis, Ration A contained 2.46 percent nitrogen and 0.062 percent sulfur, while Ration B contained 2.47 percent nitrogen and 0.705 percent sulfur. The lambs were fed and watered twice daily. The pens were bedded with shavings. When it was observed that the lambs were pulling and consuming wool from each other, they were penned separately.

Since the lambs were pair-fed on the basis of equal feed intake, the lamb eating the lesser amount determined the level of intake within each pair. The lambs on the sulfur-deficient ration limited the level of feed intake in each case.

Sulfur and nitrogen balances were conducted for three periods during the study. Collection of feces and urine was made during a 10-day period (for the third period lambs, 3, 4, 5, and 6 were on a seven-day collection period) following a similar period on a near-constant feed intake. Feed and dried fecal samples were oxidized in a Parr oxygen

bomb for total sulfur analysis, the sulfur being measured as barium sulfate. Urine samples were oxidized with the Benedict-Dennis reagent (Hawk *et al.*, 1949), before precipitation of sulfate as the barium salt. Nitrogen was determined by the Kjeldahl-Gunning-Arnold method (A.O.A.C., 1940).

At the beginning of the experiment, wool was clipped over an area approximately 5 inches square from the right shoulder of each lamb. At intervals during the test period, to obtain an estimate of the amount and composition of the wool growth, wool was clipped from sections of the shoulder area using an Oster small-animal clipper. To obtain the weight of clean wool and samples for chemical analysis,

TABLE 1. COMPOSITION OF BASAL RATION

Ingredient	Percent
Starch	41.4
Cerelose	24.5
Wood flock	10.0
Wheat straw	10.0
Minerals [1]	5.0
Corn oil	3.0
Urea	4.0
Gelatin	2.0
Choline chloride	0.1
Vitamins A. D, E, thiamine and riboflavin [2]	....
	100.0

[1] Carbonate mineral mixture of Thomas *et al.* (1951).
[2] Vitamins—each lamb was given weekly 1 gm. of vitamin A–D oil solution and synthetic E, containing 30,000 I.U. vitamin A, 2,100 I.U. vitamin D and 70 mg. vitamin E. Thiamine hydrochloride and riboflavin were incorporated in the ration at the rate of 1 mg. each per pound.

the clipped wool was washed three times in distilled water at 45° C. using neutral soap, and was rinsed four times in 45° C. distilled water.

One pair (lambs 5 and 6) was sacrificed at the 87th day of the experiment for histological study. At that time No. 5, the sulfur-deficient lamb, was very weak and dull, had a poor appetite, and exhibited excessive lacrimation and profuse salivation; the pair mate, No. 6, on the other hand, appeared strong, alert and had a good appetite.

Sections of the following tissues in each lamb were studied histologically: liver, kidney, spleen, skin, and lung. The heart muscle of No. 5 and skeletal muscle of No. 6 were also studied.

These tissues were fixed in Bouin's solution and imbedded in paraffin by the usual histological technique. The slides were stained with Harris' hematoxylin and eosin.

Results and Discussion

Figures 1 and 2 give a comparison, within each pair, of the average nitrogen and sulfur retentions, respectively, of the lambs during the three periods. Pair No. 4 was on experiment only for the first period which may account for the greater average retention of nitrogen by the basal lamb. For detailed data obtained from the nitrogen and sulfur balances see table 2.

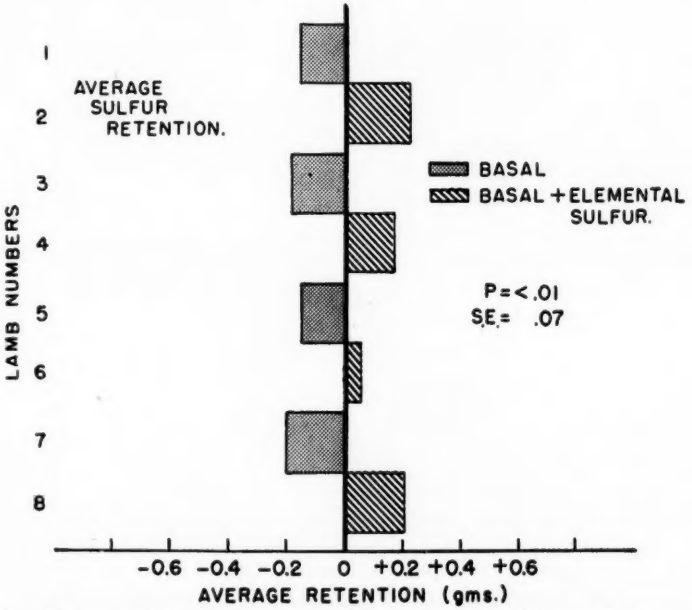

Figure 1. Effect of elemental sulfur on average sulfur retention.

The 10 observations in the three periods were combined and treated statistically as one treatment by the *t*-test ("Student," 1908). For Period I the sulfur-deficient lambs were all in negative sulfur balance, whereas all the sulfur-fed lambs were in positive sulfur balance. The nitrogen balances were not consistently in favor of either group. For Period II, the sulfur-deficient lambs were all in negative sulfur balance. The sulfur-fed lambs were in positive sulfur balance except one, No. 4, which showed a 0.13 gm. negative balance, and its pair mate, No. 3, which was in a slightly less negative balance of 0.10 gm. The sulfur-deficient lambs were all in negative balance for nitrogen.

TABLE 2. NITROGEN AND SULFUR BALANCE DATA [1, 2]

Lamb No.	Daily Values for Intake	Excretion: Feces	Excretion: Urine	Retention
	gm.	gm.	gm.	gm.
Nitrogen Balances				
Period I				
1	7.20	1.64	6.39	−0.83
2	7.70	1.65	5.61	0.44
3	7.68	1.70	6.95	−0.97
4	7.88	1.90	5.73	0.25
5	7.07	1.59	6.39	−0.91
6	6.60	1.57	6.15	−1.12
7	7.85	1.92	5.68	0.25
8	7.88	1.54	6.54	−0.20
Period II				
1	3.99	0.83	3.54	−0.38
2	4.00	1.34	2.04	0.62
3	4.55	1.28	5.04	−1.77
4	5.00	1.22	4.44	−0.66
5	9.01	2.05	7.03	−0.07
6	9.05	2.05	6.76	0.24
Period III				
1	4.95	0.98	3.43	0.54
2	4.97	1.20	3.21	0.56
3	4.91	1.22	4.03	−0.34
4	4.93	0.93	3.43	0.57
5	4.68	0.96	5.93	−2.21
6	6.38	1.98	4.88	−0.48
			Average sulfur [3]	+0.022
			basal	−0.669
Sulfur Balances				
Period I				
1	0.18	0.34	0.15	−0.31
2	2.20	1.25	0.67	0.28
3	0.19	0.37	0.19	−0.37
4	2.25	1.35	0.74	0.16
5	0.18	0.33	0.14	−0.29
6	1.88	1.06	0.67	0.15
7	0.20	0.30	0.10	−0.20
8	2.25	1.31	0.74	0.20
Period II				
1	0.10	0.11	0.09	−0.10
2	1.14	0.66	0.33	0.14
3	0.11	0.14	0.07	−0.10
4	1.43	0.90	0.66	−0.13
5	0.23	0.17	0.09	−0.03
6	2.58	1.83	0.74	0.01
Period III				
1	0.13	0.12	0.06	−0.05
2	1.42	0.76	0.40	0.26
3	0.12	0.15	0.06	−0.09
4	1.41	0.48	0.45	0.48
5	0.12	0.10	0.16	−0.14
6	1.82	1.39	0.41	0.01
			Average sulfur [4]	+0.156
			basal	−0.168

[1] Lambs 7 and 8 were removed from experiment prior to Period II.
[2] Odd-numbered lambs were on basal ration, even on basal plus elemental sulfur.
[3] $P=0.015$, S.E.$=0.229$.
[4] $P<0.01$, S.E.$=0.07$.

Lambs 2 and 6 were in positive nitrogen balance. Lamb No. 4 was in less negative nitrogen balance than its pair mate. For Period III the sulfur-supplemented lambs retained more nitrogen and sulfur than their pair mates.

The effect of elemental sulfur on maintenance and wool growth

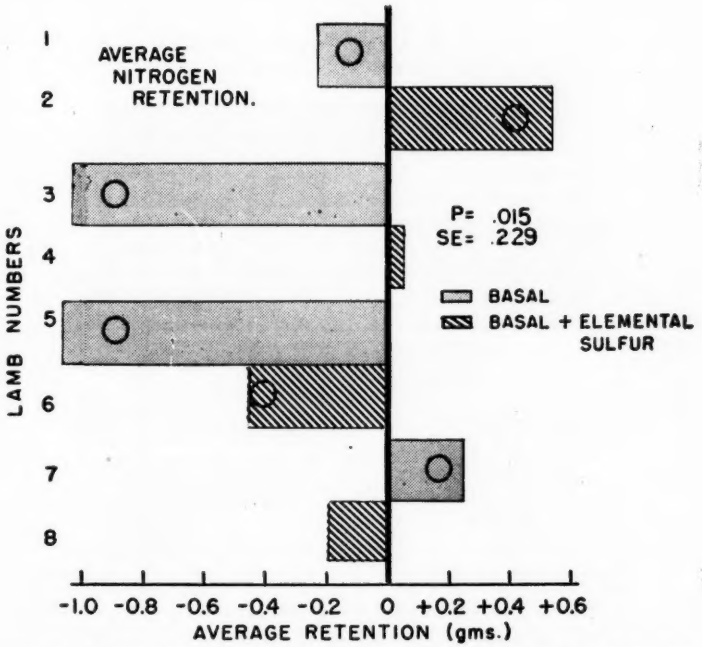

Figure 2. Effect of elemental sulfur on maintenance and wool growth. The first member in each pair received the low-sulfur, basal ration, while the second member received the basal ration plus sulfur.

is presented in figure 3. The data in table 3 show the influence of elemental sulfur on wool growth and composition. In all four pairs wool growth was favored by the supplemented ration, but the differences were not significant statistically. The nitrogen and sulfur composition was not altered by sulfur supplementation; however, the nitrogen content, expressed as percentage of dry wool, was less in each case at the termination of the trial than at the beginning. There was no difference in the sulfur content expressed as percent of the dry wool per 16 mgm. of nitrogen.

Wool growth occurred even though the lambs were losing weight, the growth being greater in the supplemented lambs. This is in accord with the data of: (1) Thomas *et al.* (1951) who reported that growth of wool appears to have a greater priority on the nutrients of the metabolic pool than does tissue growth or maintenance; (2) Rimington and Bekkar (1932) who proposed that the intestinal flora

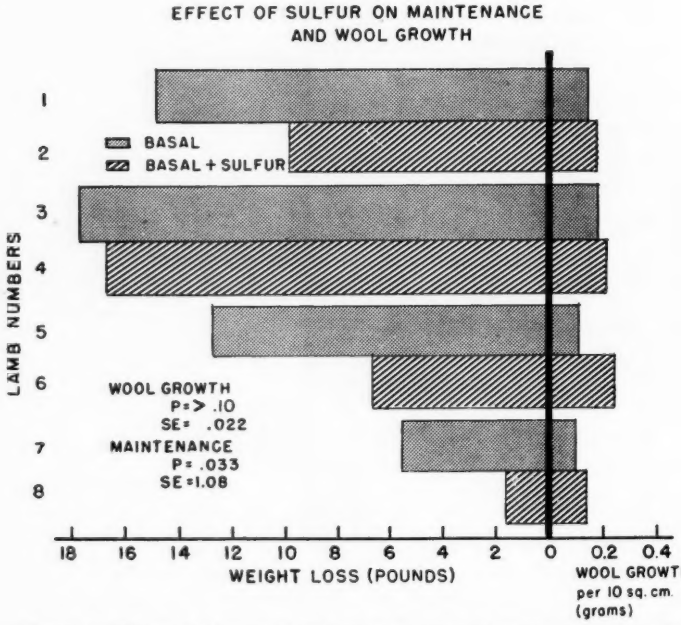

Figure 3. Effect of elemental sulfur on average nitrogen retention. The first lamb in each pair received the low-sulfur, basal ration, while the second member received the basal ration plus sulfur.

may be able to synthesize inorganic sulfur into organic form which can be utilized by the sheep in the synthesis of body tissues and keratins; and (3) Hale and Garrigus (1953) who showed that radioactive elemental sulfur when fed to a lamb appeared in the wool.

Loss of wool was evident in the sulfur-deficient group by the 27th day of the experiment but did not occur at any time among the supplemented animals. The lambs were not observed to be eating wool from themselves, but the loss of wool progressed throughout the

TABLE 3. INFLUENCE OF SULFUR ON WOOL GROWTH AND COMPOSITION [1]

Weight of wool (gm./10 sq. cm.)[2]									
No. of lamb	1	2	3	4	5	6	7	8	Average
81 days	0.142	0.172	0.176	0.202	0.100	0.219	0.071	0.112	.....
Nitrogen content (% of dry wt.)[3]									
Initial [4]	16.4	16.0	16.2	16.4	15.8	16.2	16.0	16.1	16.14
81 days	16.0	15.7	15.7	15.9	15.5	16.0	15.9	15.8	15.81
Sulfur content (S %/16 mgm. N)									
Initial [4]	3.5	3.5	3.6	3.5	3.4	3.6	3.5	3.6	3.53
81 days	3.6	3.8	3.4	3.5	3.6	3.3	3.7	3.6	3.56

[1] Wool growth was determined for lambs 7 and 8 for only 51 days.
[2] P=>0.10 in favor of sulfur-supplemented lambs. S.E.=0.022.
[3] P=<0.01 in favor of initial over 81 days. S.E.=0.049.
[4] Prior to feeding of experimental ration.

experiment. Figure 4 shows the general appearance of a typical pair at the 83rd day of the experiment.

At the 51st day of the trial No. 7 died. Numbers 3 and 5 were sacrificed for autopsy after 87 and 88 days, respectively. These lambs were very weak and dull, had poor appetites, and showed a loss of wool. Numbers 3 and 5 showed excessive lacrimation and No. 5 profuse continuous salivation starting from the 33rd day of the trial. The pair-mates, in contrast, were alert and strong, had good appetites and showed no loss of wool.

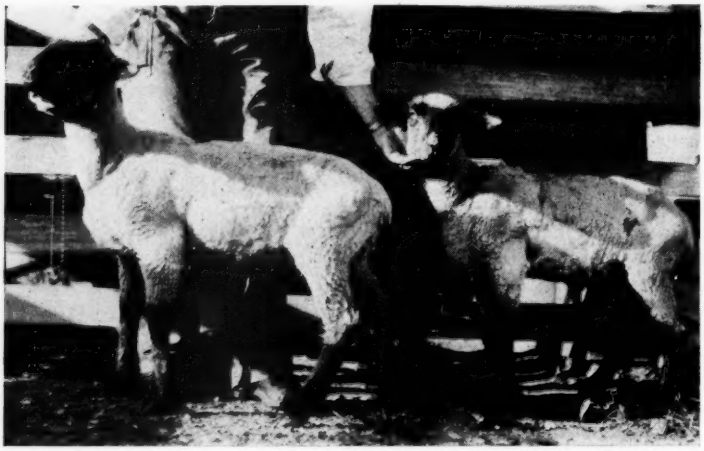

Figure 4. Pair No. 3 after 83 days on experiment. The basal lamb, No. 5, is to the right, and the pair-mate, No. 6, is to the left.

After 57 days of the experiment the eyes of each remaining lamb were examined. The eyes of the basal-fed lambs were classified from slightly cloudy to very cloudy in appearance. All of the sulfur-supplemented lambs were classified as having clear eyes. The eyes of No. 7 were noted to be cloudy before it died, while those of its pair-mate were clear.

Upon the death of No. 7, the feed intake of No. 8 was gradually increased to determine if gains could be made on the ration supplemented with elemental sulfur. This lamb was soon consuming 2.4 pounds per day with no feed refusals. A 10-pound gain was observed over a 33-day period.

A comparison of the growth curves of the lambs of each pair is shown in figure 5.

Body growth data, presented in table 4, show that the sulfur-fed lambs came closer to maintaining their weight than the basal-fed lambs ($P=0.033$). All the lambs lost weight due to the low feed intake which was limited by the basal lamb in each case.

Histopathological examination showed the following abnormalities in the tissues studied:

The liver cells of No. 5 and 6 were somewhat vacuolated, that of No. 6 even more than of No. 5.

The heart of No. 5 showed considerable degeneration of muscle fibers and proliferation of fibroblasts.

The skeletal muscle of No. 6 showed some proliferation of the lymphocytes and also degeneration of muscle fibers.

TABLE 4. BODY WEIGHT CHANGES

Lamb No.	1	2	3	4	5	6	7	8
Days on trial	93	93	88	88	87	87	56	56
Initial wt., lb.	47	44	47	49	52	45	38	37
Final wt., lb.	32	34	29	32	39	38	32	35
Loss in wt., lb.[1]	15	10	18	17	13	7	6	2

[1] $P=0.033$ sulfur-supplement over basal, S.E. $=1.08$.

The spleen of No. 5 had a marked extracellular hematogenous pigment; however, this was not believed to be due to the sulfur deficiency.

The kidneys, skin, and lung tissues of No. 5 and 6 appeared normal and the spleen of No. 6 was normal.

Summary

The paired feeding technique was used to show that elemental sulfur can be used by sheep to supply partially the dietary needs of sulfur when added to a low-sulfur ration where the major nitrogen source is urea. Utilization of feed nitrogen was definitely improved by the additional of elemental sulfur.

Lambs receiving elemental sulfur retained more nitrogen ($P=0.015$) and more sulfur ($P<0.01$), their wool growth was increased ($P>0.10$), and they came closer to maintaining their weight than those on the basal ration ($P=0.033$).

The following symptoms were observed in the sulfur-deficient lambs:

poor appetite, loss of wool, excessive lacrimation, profuse salivation, dullness, weakness, cloudy eyes, emaciation, and death.

Although histological abnormalities occurred in the liver of Nos. 5 and 6, the heart muscle of No. 5, the skeletal muscle of No. 6, and the spleen of No. 5, no definite conclusions can be drawn because those abnormalities found could be due to some complicating condition other than sulfur deficiency.

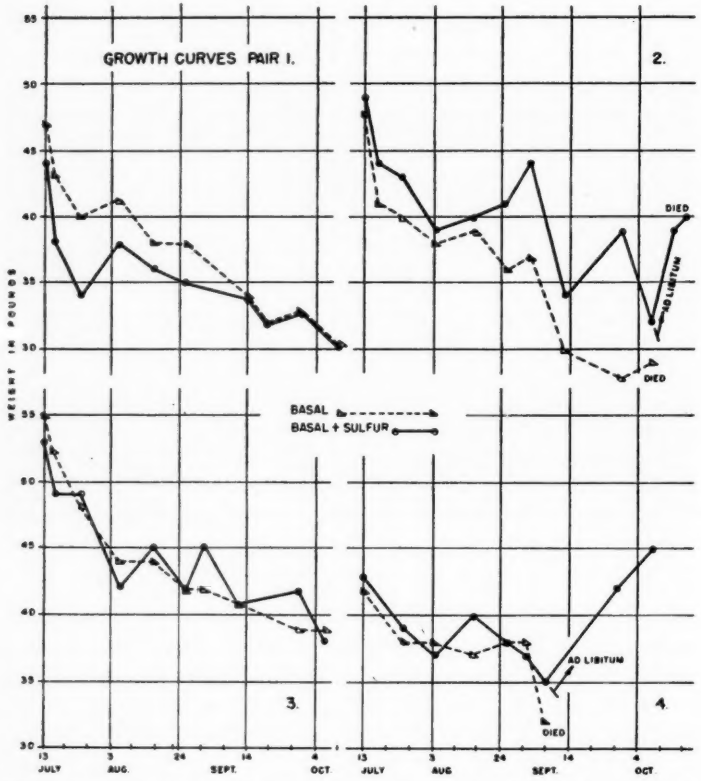

Figure 5. Comparison of the growth curves of the lambs within each pair.

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SYNTHESIS OF CYSTINE IN WOOL FROM ELEMENTAL SULFUR AND SULFATE SULFUR [1, 2]

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University of Illinois [4]

THE data in the literature do not clearly indicate the value of inorganic sulfur supplements in ruminant rations. Recently Garrigus *et al.* (1950) obtained data using a low-sulfur, semipurified ration which suggested that elemental sulfur could be utilized by sheep as measured by weight gains and wool growth. Block and Stekol (1950) showed that when $Na_2S^{35}O_4$ was administered orally to a cow the S^{35} appeared in the cystine and methionine of the milk proteins. Block *et al.* (1951) confirmed those data using a goat and also demonstrated that when $Na_2S^{35}O_4$ was administered orally to a ewe the proteins of the rumen contents contained S^{35}.

The purpose of this experiment was to determine if elemental sulfur and sulfate sulfur could be utilized in the production of wool proteins using S^{35} as a tracer.

Experimental

Two yearling wethers each weighing 120 pounds were used in this study. One wether received elemental sulfur^{35} and the other received $Na_2S^{35}O_4$. The two wethers are referred to as the elemental sulfur wether and the sulfate wether. The wethers were placed in individual feeding stalls and fed 4 pounds of corn silage daily and given daily the supplement listed in table 1. Steamed bonemeal and iodized salt were supplied free-choice.

It was thought that if a stable isotope of each sulfur compound to be tested was fed for a period of at least 30 days prior to the test period, adaptation of the rumen microorganisms for maximum conversion of the inorganic sulfur would occur. After being on the above ration for 69 days, the sulfate wether was placed in a metabolism crate and the administration of the $Na_2S^{35}O_4$ was started. Seven millicuries of the radioactive compound were given the wether in five equal doses extending over a 3-day period. The radioactive solution

[1] These investigations have been conducted under authorization from the U. S. Atomic Energy Commission, Oak Ridge National Laboratory, Oak Ridge, Tennessee. Serial Nos. 8286 and 8287.

[2] This is the third paper in a series on sulfur in sheep nutrition.

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was given orally by the use of a small rubber syringe. The elemental sulfur wether was placed in a metabolism crate after being on the above ration for 111 days and fed the elemental sulfur^{35}. A total of 7 millicuries were administered in five doses extending over a 3-day period. The test material was sprinkled over a small amount of silage to assure total consumption of the elemental sulfur^{35}. Daily urine samples were collected for determination of radioactivity. A sulfur balance was not made due to lack of facilities available for preparation of samples for counting. Urine samples were ashed according to the method of Benedict (1909) and the sulfate precipitated as benzidine sulfate. The precipitate was collected with a Tracerlab filtering funnel so that all pads to be counted had the same diameter.

TABLE 1. SUPPLEMENT FED DAILY TO WETHERS RECEIVING CORN SILAGE

Elemental Sulfur Wether	Gms.	Sulfate Wether	Gms.
Elemental sulfur	7	Na_2SO_4	7
Urea	3	Urea	3
Trace mineralized salt	6	Trace mineralized salt	6
Cerelose	100	Cerelose	100
Soybean oil meal	100	Soybean oil meal	100

At the beginning of the test period the wool was clipped from the right thigh of each wether. Fourteen days later the new growth was clipped for determination of S^{35}. The scoured wool was ashed by the method of Barritt (1934), and the sulfur was precipitated as benzidine sulfate. The precipitate was collected as described above. Blood samples were taken on the 14th day of the test period, and the serum proteins were precipitated with 10 percent trichloroacetic acid. The precipitate was ashed with nitric and perchloric acid and the sulfur precipitated as benzidine sulfate. Counting was done with a Q-gas counter and all radioactive values reported have been corrected for background count.

Results and Discussion

Only two animals were used in this experiment due to the research technique employed. However, it is believed that the following data do give a tentative basis regarding the utilization of inorganic sulfur by ruminants.

Table 2 summarizes the results of the counts observed in the urine

TABLE 2. RADIOACTIVITY OF URINE FROM WETHERS RECEIVING ELEMENTAL SULFUR^{35} AND $Na_2S^{35}O_4$

Day After Administration of Test Material	Elemental Sulfur Wether		Sulfate Sulfur Wether	
	Observed Counts per Min.	Urine Volume ml.	Observed Counts per Min.	Urine Volume ml.
1	7094	.2	20732	.2
2	15675	.2	22530	.2
3	14503	.2	22486	.2
4	10489	.5	36920	.2
5	5495	.5	19349	.2
6	1565	.5	5920	.5
7	777	.5	3778	.5
8	403	.5	1856	.5
9	408	.5	1108	.5
10	379	.5	1350	.5
11	855	1.0	1014	.5
12	183	1.0	1481	.5
13	471	2.0	854	.5
14	456	2.0	602	.5
15	292	2.0	820	1.0
16	204	2.0	949	1.0
17	121	2.0	848	1.0
18	...	...	628	1.0
19	...	...	116	1.0

samples. The radioactivty of the urine from the sulfate wether was higher than that from the urine of the elemental sulfur wether. The urine values do not give an estimation of the conversion of either compound to amino acid sulfur.

The radioactivity of the wool samples is recorded in table 3. These data show that sulfur from each of the inorganic forms appeared in

TABLE 3. RADIOACTIVITY OF WOOL FROM WETHERS RECEIVING ELEMENTAL SULFUR^{35} AND $Na_2S^{35}O_4$

Wether	Sample No.	Scoured Wool Weight	Observed Counts per Min.	Specific Activity per Mg. Sulfur [1] Counts per min.
		gms.		
Elemental Sulfur	1	.0625	618	1800±110
	2	.0879	659	1868±123
Sulfate	1	.0873	7489	15320±870
	2	.1027	7247	14865±846

[1] The specific activity values are corrected for self-absorption and decay.

the wool. The specific activity of the sulfur in the wool of the sulfate wether was approximately eight times that in the wool of the elemental sulfur wether. For demonstration as to the exact location of the radioactive sulfur, a sample of each wool was hydrolyzed for 24 hours in 6N HCl. The hydrolysates were then decolorized and small aliquots were placed on Whatman No. 1 paper strips for chromatographing. The chromatograms were developed with phenol saturated with water. Control strips using cystine, methionine, and a $Na_2S^{35}O_4$ solution were also run. Exposure of the developed chromatograms to X-ray film revealed that the cystine from both wool hydrolysates contained S^{35}. The cystine had an Rf of 0.08(0.05–0.10). A control strip showed that when small amounts of $Na_2S^{35}O_4$ were added only slight diffusion occurred from the point of application. However, no spot appeared on the film corresponding to the methionine spot on the chromatogram. In all probability this was due to the fact that wool contains only 0.4 percent methionine whereas it contains 13 percent cystine (Marston, 1946). Attempts to concentrate the methionine of wool samples were not successful.

The benzidine sulfate precipitate from 10 ml. of blood serum gave counts for the sulfate and elemental sulfur wether of 9321 and 613, respectively.

This experiment demonstrates that elemental sulfur can be synthesized into protein sulfur by sheep and confirms the findings of Loosli *et al.* (1949), Block and Stekol (1950), and Block *et al.* (1951) that ruminants are able to synthesize inorganic sulfate into protein sulfur. The difference in specific activity of the sulfur in the wool from the two experimental wethers may be suggestive of the relative efficiency of the two inorganic sulfur compounds used.

The inorganic sulfur is probably synthesized into protein sulfur by the rumen microorganisms. However, Machlin *et al.* (1952) have shown that the cystine of egg protein from eggs laid by hens injected with radioactive sulfate contains S^{35}. This suggests that routes of protein synthesis other than by rumen microorganisms from inorganic sulfur may be present.

The application of the information gained from the above data with ruminants cannot be estimated at the present time. Certainly it might be expected that the addition of inorganic sulfur to a ruminant ration low in sulfur would prove beneficial. Very little information is available on the sulfur content and ratio of nitrogen to sulfur in feedstuffs. The Oregon Agricultural Experiment Station (1950) and

Weir and Rendig (1952) report that lambs fed sulfur-fertilized alfalfa hay made more rapid gains than those fed unfertilized alfalfa hay. The effect of adding inorganic sulfur to rations in which part of the protein has been replaced by urea has been studied very little and no broad conclusions can be stated from the results obtained. The value of inorganic sulfur in ruminant rations needs further study.

Summary

Synthesis of cystine in the sheep from elemental and sulfate sulfur has been shown using S^{35} as a tracer.

Although the data obtained in this experiment are limited, the $sulfur^{35}$ activity of the wool suggests that sulfate sulfur may be better utilized for the synthesis of cystine than is elemental sulfur. This may in part be due to the difference in solubility of the two compounds.

The possible significance of the findings in relation to ruminant nutrition is briefly discussed.

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THE EFFECT OF RATION ON THE RIBOFLAVIN, NIACIN, AND VITAMIN B_{12} CONCENTRATION OF THE BLOOD AND MILK OF SHEEP[1]

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THE effect of ration on the synthesis of B-complex vitamins in the rumen has been studied quite extensively and reports may be found in the literature regarding their concentration in the rumen of both cattle and sheep. The concentration of these vitamins in the blood and milk of sheep is reported by Ray *et al.* (1947) and Pearson and Darnell (1941). Couch *et al.* (1950), Collins *et al.* (1951), and Harper *et al.* (1951) have reported the vitamin B_{12} content in the blood and milk of sheep.

No information is available regarding the concentration of these vitamins in the blood of the newborn and the infant lamb. The importance of the B-complex vitamins in the nutrition of the infant lamb is of considerable significance, as the lamb in the early part of its life depends solely upon its dam's milk to meet these vitamin requirements.

The purpose of this study was to observe the effect of the ration on the concentration of riboflavin, niacin, and vitamin B_{12} in the blood and milk of ewes and their lambs, and also on the placental transfer of these vitamins.

Materials and Methods

Experiment I. Riboflavin and Niacin

Forty grade Hampshire ewes of similar age, weight, and condition were selected for study. They were divided into four groups and fed the following rations during the latter part of gestation and through lactation. The two variables used in formulating the rations were protein (soybean oil meal) and minerals (Ca, P, I, Fe, Cu, Co, and Mn), since these two classes of nutrients are the most variable in practical rations. The nutrient intake which was kept constant with each ration met the requirements of pregnant and lactating ewes

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station, Madison.

[2] Departments of Animal Husbandry and Biochemistry. This work was supported in part by the Wisconsin Alumni Research Foundation.

according to Morrison standards. The two groups of ewes fed no protein supplement (I and II) still received a ration which met the requirements for digestible protein (table 1).

Ewes were bled at parturition and later at 6 weeks, and milk samples were collected at parturition, 5 days, 10 days, and at 6 weeks. Blood samples from all lambs were collected at birth and later at 6 weeks. During this period blood samples were also collected at the following intervals: 24 and 48 hours, 5 days and 10 days. Lambs bled at 24 hours after birth were bled again at 5 days and those at 48 hours were bled again at 10 days. The total number of lambs involved in the experiment was 52.

Blood samples collected in oxalated tubes were divided into 5 ml.

TABLE 1. RATIONS FED EXPERIMENTAL EWES

Ration Components	Groups: Daily Intake, Lb./Ewe/Day			
	I	II	III	IV
Fair alfalfa hay	2.0	2.0	4.0	4.0
Oat straw	2.0	2.0	...	...
Gr. yellow corn	1.25	1.25	1.0	1.0
Soybean oil meal	...	...	0.25	0.25
Bone meal	...	0.014	...	0.01
Mineralized salt	...	Free choice	...	Free choice
Iodized salt	Free choice	...	Free choice	...
N.R. 1:	8.6	8.6	4.5	4.5

aliquots and kept frozen until analyzed. Riboflavin was determined in the blood according to the method of Strong *et al.* (1941) and niacin according to the method of Krehl *et al.* (1943). Milk samples were given the preliminary treatment suggested by Strong and Carpenter (1942) for the determination of both riboflavin and niacin. Assay procedures were followed as described in Methods of Vitamin Assay (1947). Freezing of samples did not affect the riboflavin and niacin content of either the blood or milk.

Experiment II. Vitamin B_{12}

The following year the same ewes were divided into four groups and fed the rations shown in table 2. The purpose of this experiment was to observe the effect of mineral supplementation on the concentration of vitamin B_{12} in the blood and milk of ewes and their lambs.

The ewes were bled at parturition and at 8 weeks. Milk samples

were collected at 10 days in addition to the above time intervals. The lambs were bled at birth, 10 days, and at 8 weeks. The samples were refrigerated after collection and analyzed within 12 hours.

Diluted whole blood was analyzed according to the method of Thompson *et al.* (1950) using *L. leichmannii* ATCC 4979. The only modification made in the assay medium was that of doubling the amount of sodium acetate. Milk samples were given the preliminary treatment (Strong and Carpenter, 1942) due to the fact that direct analysis of the diluted colostrum samples yielded significantly larger values compared to the recovery samples. After the preliminary treatment the recoveries obtained ranged from 96 to 85 percent. The

TABLE 2. RATIONS FED EXPERIMENTAL EWES

Ration Components	Groups: Daily Intake, Lb./Ewe/Day			
	I	II	III	IV
Fair alfalfa hay	3.5	3.5	3.5	3.5
Oat straw	0.5	0.5	0.5	0.5
Gr. yellow corn	1.0	1.0	1.0	1.0
Bone meal	...	...	0.01	0.01
Cobaltized salt (0.0055% Co)	...	Free choice	Free choice	...
Iodized salt	Free choice	...	...	Free choice
N.R. 1:	5.8	5.8	5.8	5.8

autoclaving time was modified—7 minutes instead of 15. The titration method was used in all the analyses.

The cobalt content of hay was analyzed according to the method of Kidson and Askew (1940).

Results and Discussion

Ration did not affect the concentration of riboflavin and niacin either in the blood or milk. Data analyzed statistcally did not reveal any significant difference. Therefore all analyses were combined in reporting the concentration of these vitamins.

The riboflavin content of the ewes' blood averaged 0.4 γ/ml. at parturition. Six weeks later it had decreased to 0.22 γ/ml. The niacin content of the ewes' blood was 25 times greater in concentration compared to riboflavin at parturition and remained almost constant with an average of 10 γ/ml. through 6 weeks.

The riboflavin content of colostrum averaged 25.8 γ/ml. which was

7–8 times greater than that in the normal milk. A sharp decline in the riboflavin content occurred 5 days after parturition; thereafter the decline was gradual and at 6 weeks the riboflavin value was 4.9 γ/ml. The reverse was true for niacin, 2.3 γ/ml. in colostrum rising to 6.6 γ/ml. in milk 6 weeks later.

Both riboflavin and niacin decreased in the lambs' blood from 0.5 and 20 γ/ml. at birth to 0.3 and 12 γ/ml. at 6 weeks, respectively.

In contrast to vitamin A in ewes' colostrum, the excessive riboflavin content did not affect the vitamin blood level of lambs. The only explanation that can be offered in this regard is that riboflavin may have been stored in the body for subsequent use. Niacin was 2–3

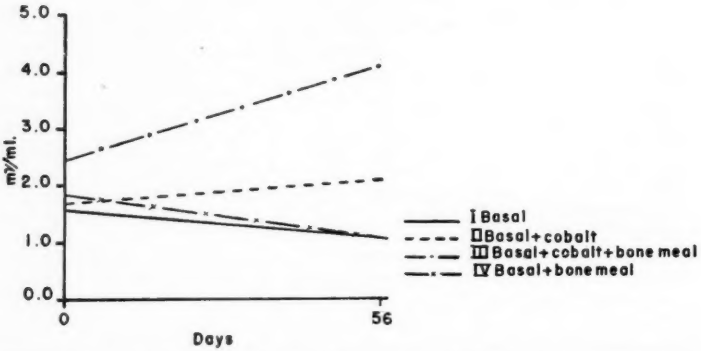

Figure 1. Effect of ration on the concentration of vitamin B_{12} in the blood of ewes.

times lower in colostrum than in the normal milk. The difference in the behavior of riboflavin and niacin may be due to the fact that niacin is synthesized in the animal body (Snell and Wright, 1950). In addition the requirement for niacin in the lamb probably increases with growth. In the absence of any synthesis of riboflavin in the animal body, it is apparent that this vitamin is required as such and the storage of this vitamin as pointed out earlier seems plausible in view of the decrease in the concentration of this vitamin with advanced lactation. There was placental transfer of these vitamins in the pregnant ewes.

The results of the vitamin B_{12} analyses are presented in figures 1, 2 and 3. The vitamin B_{12} content of the blood of the ewes at parturition was between 1.5 and 2.0 mγ/ml. for all the groups except lot III

which showed a slightly higher activity. Subsequent to parturition, an increase in the vitamin B_{12} content of the blood was observed in lot II (Co) and lot III (Co + bone meal), whereas lot I (basal) and lot IV (bone meal) showed a very slight decrease (figure 1). Lot III at 8 weeks was significantly higher ($P<0.05$) as compared with lots I and IV.

In figure 2 is shown the vitamin B_{12} concentration in the lambs' blood at birth and in the subsequent period of 8 weeks. Marked increase in the vitamin B_{12} content occurred in the case of cobalt-

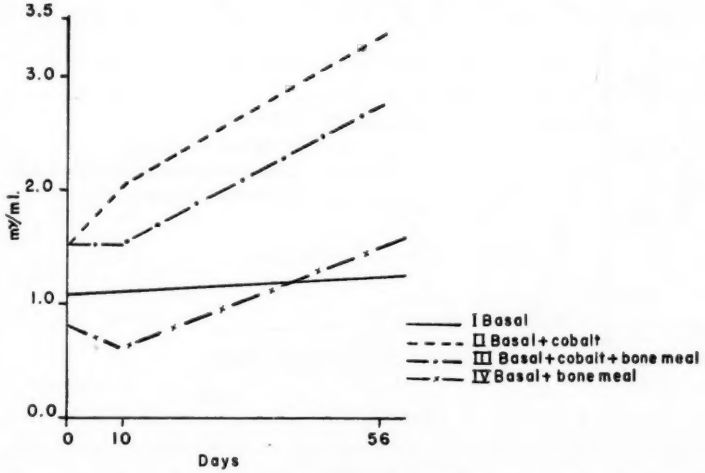

Figure 2. Effect of ration on the concentration of vitamin B_{12} in the blood of lambs.

supplemented groups. Lots I and IV showed a very slight increase during the period; however, the increases were not significant statistically.

The vitamin B_{12} content of colostrum was 3–4 times higher in the cobalt-supplemented lots than in lots I and IV. The differences were not significant due to greater individual variation in the cobalt-supplemented groups. Colostrum samples from lot III exhibited a comparatively greater uniformity in the vitamin content than lot II. The values in lot II ranged from 3 to 59 mγ/ml. In lots II and III (cobalt-supplemented groups) a marked decrease in the vitamin content was observed at 10 days after parturition which continued until

8 weeks, whereas lots I and IV showed only a gradual decrease during the same period. Lot III at 8 weeks was significantly higher ($P<0.05$) as compared with lots I and IV (figure 3).

Couch *et al.* (1950) reported 0.7 mγ/ml. vitamin B_{12} activity in sheep blood, but failed to indicate the cobalt content of the ration. Sreenivasamurthy *et al.* (1950) observed the vitamin B_{12} activity of the milk of the cow, sheep, and goat to be 1.45, 14.0, and 0.85 mγ/ml., respectively. These workers also did not mention the cobalt content of the ration. Moreover, on the basis of the data obtained in this

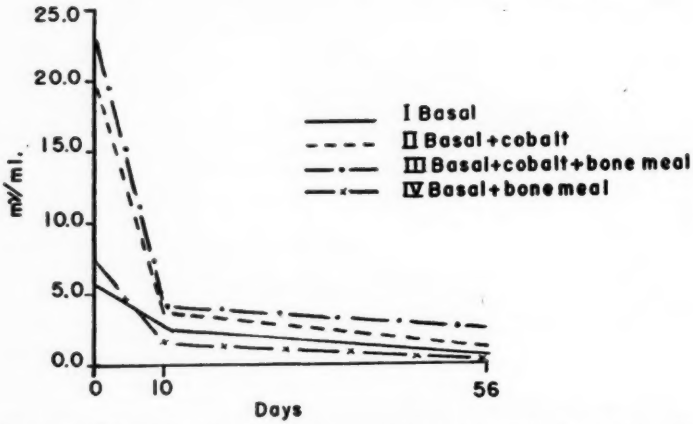

Figure 3. Effect of ration on the concentration of vitamin B_{12} in the milk of ewes.

study, it is felt that not only the cobalt content of the ration is important but, in the case of milk analysis, the stage of lactation as well. Harper *et al.* (1951) have analyzed ewes' milk during the third and fourth month postpartum and reported values for vitamin B_{12} as 1.38, 7.89, and 9.58 mγ/ml. in groups maintained on iodized salt, cobaltized salt, and trace-mineralized salt, respectively. No preliminary treatment was given to the milk samples before analyzing for vitamin B_{12}. Similarly the values obtained by Collins *et al.* (1951) for vitamin B_{12} in the milk of cow, sheep, and goat (6.6, 1.4, and 0.12 γ/liter, respectively) were the result of direct analysis of milk. The preliminary treatment of milk causes a decrease in the vitamin B_{12} activity but it was believed that the values obtained in this manner were more representative of the true values.

Salt consumption during the experimental period by the four lots in numerical order was 30.0, 55.5, 66.0, and 66.0 pounds for the ewes and 2.0, 1.7, 2.0, and 1.75 pounds for the lambs, respectively. The cobalt content of hay was 0.09 p.p.m.; corn did not show any measurable activity. The calculated intake of cobalt was approximately 0.7 mg./ewe/day in the cobalt-supplemented groups.

In view of the fact that lot III, which received bone meal in addition to the cobaltized salt in the ration, showed a higher concentration of vitamin B_{12} in the blood and milk of ewes at every sampling time, it was decided to test the effect of calcium and phosphorus on the synthesis of vitamin B_{12} as measured in the blood. The test was

TABLE 3. RATIONS FED EXPERIMENTAL LAMBS

Ration Components	Groups: Daily Intake/Lamb/Day			
	I	II	III	IV
Alfalfa-brome grass hay	2.0 lb.	2.0 lb.	2.0 lb.	2.0 lb.
Gr. yellow corn	0.5 lb.	0.5 lb.	0.5 lb.	0.5 lb.
Cobaltized salt (0.0055% Co)	Free choice	Free choice	Free choice	Free choice
$CaCO_3$ (440 gm./100 lb. corn)	...	2.20 gm.	...	...
NaH_2PO_4 (410 gm./100 lb. corn)	...	...	2.05 gm.	...
$Ca_3(PO_4)_2$ (454 gm./100 lb. corn)	...	...	...	2.27 gm.
Ca:P ratio	2.5:1	3.5:1	1.8:1	2.4:1

made to determine whether the beneficial effect was due to calcium or phosphorus alone or a combination of the two.

Sixteen lambs about 8 months of age were selected and fed a ration consisting of hay, ground yellow corn, and iodized salt for a period of 1 month. It was believed that during this time when all the lambs were receiving a similar ration, their vitamin B_{12} synthesis should become reasonably standardized. At the end of this period blood samples from all lambs were collected and analyzed for vitamin B_{12} and hemoglobin, and the animals were then divided into four groups and fed the experimental rations shown in table 3. Lot II received $CaCO_3$ in an amount which would furnish the same amount of calcium as was received by lot IV in the form of $Ca_3(PO_4)_2$, whereas lot III was supplied with NaH_2PO_4 calculated to provide the same amount of phosphorus as received by lot IV. The cobalt, calcium, and phos-

TABLE 4. VITAMIN B_{12} CONTENT OF LAMBS' BLOOD

mγ/ml.

Lot No.	Beginning of Exp.		3 Wks. on Exp.		5 Wks. on Exp.	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
I	0.78	.137(4) [1]	2.73	.178(4)	3.22	.32(4)
II	0.67	.099(4)	2.34	.37(4)	2.64	.83(4)
III	0.73	.20(4)	2.56	.55(4)	2.24	.73(4)
IV	0.89	.26(4)	2.42	.33(4)	2.76	.54(4)

[1] Numbers in parentheses denote the number of lambs.

phorus content of hay was 0.12 p.p.m., 0.8 and 0.18 percent, respectively. The lambs were bled twice during the 5 weeks' experimental period. Vitamin B_{12} and hemoglobin were determined each time. The results are presented in tables 4 and 5.

The vitamin B_{12} content of the lambs' blood at the end of the 1-month preliminary period (beginning of experiment in table) was low. During this time the cobaltized salt had been withheld. The vitamin B_{12} content increased in all groups, however, with the addition of cobaltized salt to the rations. There was no statistically significant difference between the groups at any time of analysis. The salt consumption in the four groups was 4.25, 3.50, 3.50, and 3.75 pounds, respectively. The group that showed the highest activity of vitamin B_{12} consumed more salt than the other ones as was the case in the preceding experiment.

On the basis of the data obtained in this and the preceding experiment, it is concluded that the vitamin B_{12} content of blood and milk is dependent upon the amount of cobalt ingested, and calcium and phosphorus either alone or in combination do not exert any effect. A gradual increase was observed in the hemoglobin content of the blood of all groups after the addition of cobaltized salt to the rations.

TABLE 5. HEMOGLOBIN VALUES OF LAMBS' BLOOD

gm./100 ml. blood

Lot No.	Beginning of Exp.	3 Wks. on Exp.	5 Wks. on Exp.
I	10.86	11.88	12.65
II	8.66	12.41	13.67
III	10.12	10.92	12.88
IV	8.86	10.42	12.10

Summary

Statistical analysis of the data on riboflavin and niacin content of the blood and milk of ewes and of the blood of their lambs did not reveal any significant difference caused by protein or mineral supplementation.

The riboflavin content of ewes' blood was 0.4 γ/ml. at parturition. Six weeks later it had decreased to 0.22 γ/ml. On the other hand, niacin tended to remain constant with an average of 10 γ/ml.

In the case of both riboflavin and niacin, the level in lambs' blood decreased gradually from birth to 6 weeks. The values were 0.5 and 20 γ/ml. and decreased to 0.3 and 12 γ/ml. for riboflavin and niacin, respectively.

Riboflavin was 7–8 times higher in colostrum than in normal milk. The colostrum content was 25.8 γ/ml. and it decreased to 4.9 γ/ml. at 6 weeks. The reverse was true for niacin, 2.3 γ/ml. in colostrum and 6.6 γ/ml. in milk 6 weeks later.

The vitamin B_{12} content of the blood at parturition was of the same order, approximately 1.5 γ/ml., in all groups. Following parturition, the cobalt-supplemented groups showed an increase in the vitamin B_{12} content, whereas in the unsupplemented groups a decrease was observed. Lot III (bone meal + cobalt) was significantly higher than the other groups at 6 weeks.

The vitamin B_{12} concentration of the lambs' blood at birth was approximately the same in all groups, 1 mγ/ml. Ten days after birth a marked increase in the vitamin B_{12} content was observed in the cobalt-supplemented groups. The other two groups showed very slight decrease. The marked increase in the cobalt-supplemented groups may be attributed to the high vitamin B_{12} potency of the milk of their dams.

The vitamin B_{12} content of colostrum in lots I (basal) and IV (bone meal) was 5.05 and 7.3 mγ/ml., respectively, as compared to 19.80 and 23.20 mγ/ml. in lots II and III (Co-supplemented). The latter values decreased quite markedly at 6 weeks. Lot III with 2.59 mγ/ml. was significantly higher when compared with the other groups.

Placental transfer of all these vitamins occurred in the pregnant ewes.

Supplementation with calcium and phosphorus alone or in combination did not affect the vitamin B_{12} content of blood. Hence it is believed that the vitamin B_{12} concentration in both the blood and milk is directly dependent upon the amount of cobalt ingested.

Since vitamin B_{12} is important as a growth and hemopoietic factor, supplementation with cobalt in the ration of the pregnant ewe is advised.

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DETECTION OF ESTROGENIC SUBSTANCES IN ALFALFA AND CLOVER HAYS FED TO FATTENING LAMBS [1]

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SEVERAL reports have demonstrated the presence of estrogenic substances in growing plants. In Western Australia considerable attention has been given to the estrogenic activity present in subterranean clover (Bennetts *et al.*, 1946; Curnow *et al.*, 1948; and others) which has interfered with breeding performance in sheep. In England Bartlett *et al.* (1948) and Legg *et al.* (1950) reported the presence of estrogens in certain grasses and pointed out that the increased milk secretion often noted during the spring pasture season might be due to the estrogenic effects of the grass. More recently Dohan *et al.* (1951) have shown estrogenic activity in spring rye grown in the United States. Interest in estrogenic substances in animal nutrition has been stimulated further by recent experiments showing that fattening lambs and cattle (Andrews *et al.*, 1949; Jordan, 1950; Jordan *et al.*, 1952) often make more rapid gains in body weight when the estrogenic substance stilbestrol (diethylstilbestrol) is implanted under the skin.

In a lamb feeding experiment at this Station, Culbertson *et al.* (1952) were unable to confirm the growth-stimulating effect of stilbestrol implantation noted by others. Since the control lot of lambs receiving no stilbestrol in this experiment made exceedingly good gains comparable to those of the implantation lot, it was postulated that the clover fed might have contained sufficient estrogenic activity such that no additional stimulation could be obtained with additional estrogenic activity coming from the stilbestrol implantation. This experiment was repeated using the same source of clover hay as used initially and again repeated with a third experiment (unpublished data) using alfalfa hay in which essentially no stimulation was observed from stilbestrol implantation in fattening lambs.

The purpose of this study was to determine whether the clover and alfalfa hays fed in these Iowa lamb fattening experiments contained estrogenic activity. Also preliminary information was sought concern-

[1] Published with the approval of the Director of the Iowa Agricultural Experiment Station, Ames, Iowa, as Journal Paper No. J-2232, Project 1208.

ing the nature and presence of estrogenic substances in certain other cattle and sheep feedstuffs.

Experimental Procedure

The clover hay tested was grown in the summer of 1951 and was a mixture of second-cutting red *(Trifolium pratensis)*, alsike *(Trifolium hybridum)*, and ladino *(Trifolium repens L.)* clovers in which red clover predominated. The alfalfa *(Medicago sativa)* hay was first-cutting grown on the College Farm in the spring of 1952. The overall procedure employed in testing estrogen activity consisted of three steps. Crude extracts were prepared from the hay or other feedstuff in the initial step. The second step involved purification of the crude extracts obtained. The final step consisted of measuring the response of purified extracts upon the uterine weight of immature female mice and comparing this response to responses noted with measured amounts of stilbestrol injected into control or assay mice.

Several procedures were tried in the initial extraction of the hay samples. The procedure ultimately adopted was a modification of the method used by Beck and Braden (1951). The details of this procedure can be illustrated as follows: To a 500 gm. air-dried ground hay sample, 2000 ml. of 95 percent ethanol were added followed by refluxing for 1 hour. Sufficient alcoholic sodium hydroxide was next added to make the solution equivalent to 0.5 N NaOH. Refluxing was then continued for an additional 10 minutes. After cooling, the alcoholic extract was removed by filtration and saved. The hay residue was extracted a second time using 1000 ml. of 95 percent ethanol and refluxing for 15 minutes followed by filtration. The two extracts were combined, neutralized with alcoholic sulfuric acid to pH 6.6 and allowed to stand overnight in a refrigerator. The solution was then filtered and evaporated to dryness under vacuum. This dry residue constituted the crude extract which required purification before it was suitable for feeding in satisfactory amounts or before it could be injected in appreciable amounts without being toxic to mice.

The purification consisted of allowing the dried residue to stand with 50 ml. of saturated $NaHCO_3$ and 100 ml. of ether for a few minutes before transferring to a separatory funnel. Any remaining residue that adhered to the flask was washed out with further additions of $NaHCO_3$ and ether, the washings being added to the separatory funnel. The estrogenic substances were extracted by adding another 100 ml. of ether and removing the ether portions from the separatory

funnel. These combined ether extracts were next concentrated to 50 ml. and the active fraction removed by three extractions with an equal volume of 0.1 N NaOH. This alkaline solution was acidified and extracted twice with a small volume of ether. After washing with water, the ether phase was evaporated to dryness. This dry material when placed in olive oil was found satisfactory for injection into mice at relatively high levels without difficulties from toxic substances extracted from the hay.

For the assay of these purified hay extracts, a modification of the method described by Evans *et al.* (1941) was used. In this method the potencies of the extracts were assayed by noting the increased uterine weight of immature female mice receiving injections of the extracts against those receiving standard doses of stilbestrol (0 to .04 micrograms). Immature female mice from 8 to 10 gm. in weight obtained from the Carworth Farm in New York (Strain C.F.W.) were used. During the test period they were maintained on a ration containing 74 percent corn, 20 percent dry skim milk, 4 percent corn oil and 2 percent salt mixture. The extract was injected subcutaneously at the dose of 0.05 ml. per mouse per day for 4 days. Olive oil was used to dilute the extract to any desired concentration. The animals were killed 24 hours after the last injection. The uteri were dissected out and fixed in Bouin's fluid for 24 hours. They were then dried by pressing against filter papers, the uteri further trimmed of any adhering tissues and weighed.

Results and Discussion

Estrogenic activity was found in the clover hay fed to fattening lambs. It can be seen from table 1 that an increase in uterine weight was obtained either by feeding or by subcutaneous injection of the

TABLE 1. ESTROGENIC ACTIVITY OF CLOVER HAY

Treatment [1]	No. of Mice	Uterine Wt. in Mg.	
		Avg.	Std. Dev.
Control (no injection)	45	7.7	4.1
Control (olive oil injection)	16	8.8	4.0
Feeding 2 gm. clover hay	10	7.0	2.6
Feeding crude extract from 5 gm. hay	13	12.7	4.8
Feeding purified extract from 5 gm. hay	23	13.1	5.8
Injecting purified extract from 5 gm. of hay	12	31.3	6.2

[1] Treatment was given daily for 4 days.

clover extract. However, with the purified extract, injection was far more effective than feeding. Therefore in later experiments all extracts were purified and injected subcutaneously into mice.

The results obtained by injecting different levels of this clover hay extract are presented in figure 1. Also in this figure is shown the uterine response from different levels of stilbestrol injected into comparable mice. It can be seen that the response from clover extract equivalent to 2 to 5 gm. of hay per day was essentially linear. Also it can be observed that the uterine weight response to increasing levels of stilbestrol was approximately linear up to a level of .04 micrograms. From these values 1 pound of the clover hay was estimated as containing the equivalent activity of 2 micrograms of stilbestrol.

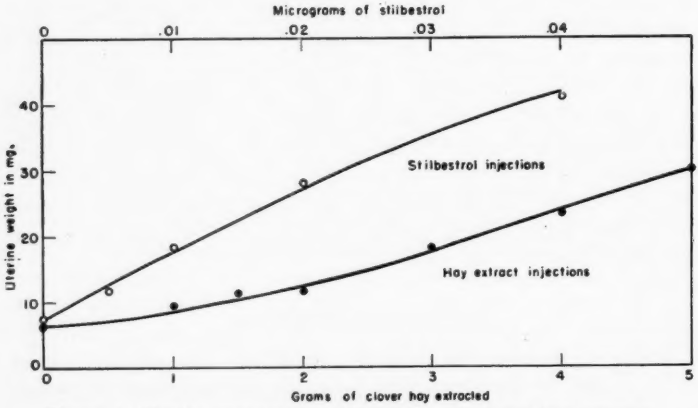

Figure 1. Uterine response to stilbestrol and clover hay extracts.

This value is about 10 times the potency found by Dohan *et al.* (1951) on spring clover and spring rye. In England the red clover tested was estimated to have a higher estrogenic activity, ranging from 8 to 16 micrograms of estradiol per pound of dry matter. The subterranean clover of Australia was found to have a potency as high as 45 micrograms of stilbestrol per pound of dry hay (East, 1950). It seems reasonable that variations in estrogenic potencies might be expected in different species of plants and in the same species of plant grown under different environmental conditions or cut at different stages of maturity.

The estrogenic activity found in first-cutting alfalfa hay (1952) fed to fattening lambs at this Station is presented in table 2 along with results obtained with various other feedstuffs.

This alfalfa hay contained less estrogenic activity (0.88 microgram stilbestrol per pound of hay) than that found in the clover hay fed to lambs (1.95 micrograms per pound). It is interesting to note in table 2 that second-cutting clover hay contained more

TABLE 2. POTENCIES OF ESTROGENIC SUBSTANCE IN FEEDSTUFFS

Feedstuffs	Dose [1]	No. of Mice	Uterine Wt. Mg.		Estimated Potency in Mcg. Stilbestrol per Lb. of Feed
			Avg.	Std. Dev.	
Control (no injection)		20	7.4	2.3	....
First-cutting clover (1951)[2]	5 gm.	6	15.1	2.6	0.87
Second-cutting clover (1952)	5 gm.	9	32.4	5.6	1.95
First-cutting alfalfa (1952)[2]	5 gm.	9	15.5	4.9	0.88
Second-cutting alfalfa (1952)	5 gm.	6	26.4	4.3	1.61
Third-cutting alfalfa (1952)	3 gm.	8	25.0	2.2	2.72
Blue grass	4 gm.	7	8.2	2.4	....
Brome grass	4 gm.	7	7.8	1.1	....
Ladino clover	5 gm.	10	8.2	2.9	....
Wheat plant	5 gm.	6	8.5	1.5	....
Soybean oil meal	5 gm.	8	6.9	2.5	....
Ground yellow corn	5 gm.	7	7.1	1.3	....
Ground wheat	5 gm.	6	7.7	1.7	....

[1] Weight of air-dry material represented by dose of extract administered daily.
[2] Hays used in lamb fattening experiments.

estrogenic activity than first-cutting clover hay. The same relationship existed in two alfalfa hays tested, namely, second-cutting was more potent in estrogens than first-cutting. The third-cutting alfalfa, however, contained the highest estrogenic activity of all the hays tested. Whether any significance can be attached to this relationship is not known at present. Legg *et al.* (1950) found that red clover cut in February had less activity than that cut in March, but the potency declined in June. Single samples of blue grass, brome grass, ladino clover and the immature wheat plant tested in this study as well

as soybean oil meal, corn grain, and wheat grain failed to show estrogenic activity.

The specific substance responsible for the estrogenic activity found in hay has not been definitely identified to date. Curnow and Bennetts (1952) indicated that the estrogenic material present in subterranean clover was largely, if not entirely, 5:7:4′ trihydroxy isoflavone (Genistein). This compound is particularly interesting from the standpoint of animal feeding since the glucoside of this compound (Genistin) is found (Walter, 1941) in relatively large amounts in soybean oil meal (0.1%). Preliminary tests in this laboratory (unpublished data) indicate that genistein prepared from genistin isolated from soybean oil meal has significant estrogenic activity in mice.

The significance of estrogenic substances found in feedstuffs commonly fed to farm animals merits further study. One question of considerable importance is whether or not the amounts present in various forages are of sufficient magnitude to be either helpful or harmful to different species or different classes of a given species of farm animals. In the case of Australian sheep it has been definitely established that the amount of estrogenic substances in subterranean clover is injurious to breeding performance. Based upon the reported amounts of estrogenic substance present in subterranean clover, the present study would indicate that certain of our American hays contain appreciable amounts of estrogens, but lesser amounts than that reported in subterranean clover. No experimental studies to the authors' knowledge have been made upon impaired breeding performance of livestock when fed estrogenic hays produced in this country. However, field reports of breeding difficulties in sheep have come to the authors' attention which are suggestive that these breeding difficulties may be related to ration characteristics as yet unidentified.

Another question of potential importance is whether or not the amounts of estrogenic substances in certain hays and green forages are sufficiently high so that milk production in lactating animals is stimulated or whether growth or live weight gains in fattening cattle or sheep are stimulated. The amount of estrogenic activity furnished in the hays fed to the Iowa experimental lambs was only about one fiftieth the amount of activity needed to be effective in fattening lambs, if it is assumed that a 15 mg. stilbestrol pellet is a minimal or threshold dosage. This assumption may or may not be true since threshold levels of stilbestrol pellets in lambs have not been critically

evaluated. Also it is conceivable that plant estrogenic substances which remain active when present in the feed may have a different threshold level than those which are administered via implantation or pellets. The failure of stilbestrol implantation in fattening lambs to stimulate weight gains in the Iowa experiments, and the subsequent finding of estrogenic activity in the hay fed and tested in this study, can be regarded as circumstantial evidence that the level of estrogens found in the hays fed contributed to the excellent gains made by the lambs.

Summary

Estrogenic activity was found in clover and alfalfa hays fed to fattening lambs. Also estrogenic activity was found in other alfalfa and clover hays. This activity was assayed against standard doses of stilbestrol by noting the stimulation in uterine weights of immature female mice. Other feedstuffs tested (one sample each) which failed to show estrogenic activity were: blue grass, brome grass, ladino clover, immature wheat plants, corn and wheat grains, and soybean oil meal. The potency of the clover and alfalfa hays varied from 0.8 to 2.7 micrograms of stilbestrol activity per pound of hay tested. The possible significance in feeding practice of these levels of estrogens in hays upon breeding performance, milk production, and rate of gain in fattening animals is discussed.

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INDUCTION OF ESTRUS AND OVULATION IN ANESTRUAL EWES BY USE OF PROGESTERONE AND PREGNANT MARE SERUM [1]

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A REVIEW of the literature regarding hormonal stimulation of reproductive function in anestrual ewes establishes a number of points; namely, that ovulation can be produced fairly consistently by injection of PMS, but that estrus accompanies induced ovulation in only a low percentage of treated ewes, and that forced mating after injection with PMS rarely results in conception. The reason for failure to produce synchronous estrus and ovulation consistently is not clearly understood; however, the important role of the corpus luteum in control of the rhythmicity of the estrual cycle may shed some light on this problem.

Cole and Miller (1935) showed that during early and late anestrus ewes exhibit a semi-cyclic pattern in ovarian activity as evidenced by changes in the vaginal smear. Hammond, Jr. (1944) and Robinson (1950) have shown that there is a continual growth and regression of follicles during anestrus even though ovulation does not occur. During anestrus there is clearly a pattern of rhythmic change taking place in the ovaries and this pattern may in part explain the varied response when the ovulation stimulus is provided by injecting gonadotrophic hormone. Work at Wisconsin (Dutt and Casida, 1948; O'Mary *et al.*, 1950) has shown further that estrus and ovulation can be synchronized in a group of estrual ewes by use of progesterone. It seems appropriate to determine whether progesterone might also serve to synchronize this semi-cyclic pattern during anestrus.

That progesterone may have a stimulating effect on the release of luteinizing hormone by the pituitary gland has also been demonstrated. Everett (1943) has stimulated ovulation by appropriately-timed injections of progesterone in a strain of persistent estrual rats. Pfeiffer (1950) has shown that when progesterone is injected at the proper time ovulation is stimulated in the monkey during anovulatory summer cycles. Sawyer *et al.* (1950) have found that progesterone injection following estrogen treatment causes ovulation in the rabbit

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

without the stimulus of coitus. Hansel and Trimberger (1952) were able to hasten ovu[illegible] in dairy heifers by injecting progesterone early in estrus. Sm[illegible] (1952) has found that ovulation time in estrual ewes can be sl[illegible]ened by progesterone injection early in estrus. These reports do not agree entirely with present concepts of the mechanism for reciprocal relationship between the ovary and the pituitary gland with respect to progesterone, and therefore afford the basis for some interesting speculation.

In order to determine whether synchronous estrus and ovulation could be induced in anestrual ewes with progesterone treatment prior to treatment with PMS, 23 ewes were given five injections of progesterone at 3-day intervals followed 3 days later by PMS injection during June and July 1951. Twenty-two of the ewes came into estrus and were bred within 2 days after the PMS injection. Eleven of the ewes lambed.

The following experiment was carried out in late May and June 1952 to study further the action of progesterone in anestrual ewes.

Experimental Procedure

Seventy-two Hampshire-cross western ewes were randomly divided into eight groups of nine ewes each. Group I served as a control group; group II received one SCI [2] of 500 IU PMS [3]; group III received one SCI of 30 mg. progesterone [4] and 3 days later one SCI of 500 IU PMS; group IV received two SCI of 30 mg. progesterone at a 3-day interval followed 3 days later by one SCI of 500 IU PMS; group V received three SCI of 30 mg. of progesterone at 3-day intervals followed 3 days later by one SCI of 500 IU PMS; group VI received four SCI of 30 mg. of progesterone at 3-day intervals followed 3 days later by one SCI of 500 IU PMS; group VII received five SCI of 30 mg. progesterone at 3-day intervals followed 3 days later by one SCI of 500 IU PMS; and group VIII received only the five SCI of 30 mg. progesterone at 3-day intervals. The plan of treatment for the various groups is shown in table 1.

The series of ewes on the progesterone treatment should (1) furnish information on whether this treatment is more effective in producing synchronous estrus and ovulation during anestrus than is PMS injection alone, and (2) provide some information regarding

[2] Subcutaneous injection.
[3] Five hundred International Units of Pregnant Mare Serum in the form of the commercial preparation, Anteron.
[4] Crystalline Progesterone USP XIII in 1 ml. of corn oil.

the mode of action of progesterone. The group on progesterone alone should furnish a check on whether progesterone is effective in stimulating the pituitary gland to release luteinizing hormone above the amount normally secreted during this period of the year. The dosage of 500 IU of PMS was used since preliminary work showed this level would result in approximately a normal ovulation rate. Thirty milligrams of progesterone were injected at 3-day intervals since this was found to be as effective in synchronizing estrus during the breeding

TABLE 1. TREATMENT GROUPS FOR ANESTRUAL EWES RECEIVING SUBCUTANEOUS INJECTIONS OF PROGESTERONE AND PREGNANT MARE SERUM

Group	Date					
	May 27	May 30	June 2	June 5	June 8	June 11
I	Control	.....	.....	.....	.....	.....
II	500 IU [1]	.....	.....	.....	.....	.....
	PMS	.....	.....	.....	.....	.....
III	30 mg.[2]	500 IU	.....	.....	.....	.....
	Prog.	PMS	.....	.....	.....	.....
IV	30 mg.	30 mg.	500 IU	.....	.....	.....
	Prog.	Prog.	PMS	.....	.....	.....
V	30 mg.	30 mg.	30 mg.	500 IU	.....	.....
	Prog.	Prog.	Prog.	PMS	.....	.....
VI	30 mg.	30 mg.	30 mg.	30 mg.	500 IU	.....
	Prog.	Prog.	Prog.	Prog.	PMS	.....
VII	30 mg.	30 mg.	30 mg.	30 mg.	30 mg.	500 IU
	Prog.	Prog.	Prog.	Prog.	Prog.	PMS
VIII	30 mg.	30 mg.	30 mg.	30 mg.	30 mg.	.....
	Prog.	Prog.	Prog.	Prog.	Prog.	.....

[1] Anteron—International Units.
[2] Crystalline Progesterone USP XIII in corn oil.

season as were daily injections of 10 milligrams (unpublished data, University of Kentucky).

Treatment was started on May 27, and the experiment continued through June 19. All ewes were checked for estrus once daily through the experimental period. Those showing estrus were artificially inseminated and slaughtered 3 days later in order that the ovaries could be examined and also to determine whether fertilization had occurred in ewes in which ovulation had taken place. Sections of the uteri were also saved for histological study. Treated ewes which did not show estrus within 10 days after the end of treatment were slaughtered at that time; the ovaries were examined for the presence of corpora

lutea and the state of follicular development was recorded. On June 18 and 19 at the end of the experiment the control ewes were laparotomized and the ovaries were examined for the presence of corpora lutea, and follicular measurements were also taken.

Results

None of the control ewes (group I) came into estrus during the experimental period, and when they were laparotomized at the end of the study neither active nor waning corpora lutea were found on

TABLE 2. RESPONSE OF ANESTRUAL EWES TO INJECTION WITH PROGESTERONE, PREGNANT MARE SERUM AND PROGESTERONE FOLLOWED BY PREGNANT MARE SERUM

Group [1]	Number of Ewes in Estrus within 10 Days	Number of Ewes Ovulated	Percent of Ova Fertilized (from Ewes Showing Estrus)	Ovulation Rate	Interval from End of Treatment to Estrus
					days
I. Control	0	0	....	....	....
II. PMS	0	9	....	1.8	....
III. 1P–PMS	3	8	0(4) [2]	1.5	1–2
IV. 2P–PMS	6	9	37.5(8)	1.6	1–2
V. 3P–PMS	6	9	55.6(9)	1.6	1–2
VI. 4P–PMS	8	9	60.0(10)	1.3	1–2
VII. 5P–PMS	9	9	53.3(15)	1.7	1–2
VIII. 5P	4	5	60.0(5)	1.5	4–5

[1] Nine ewes in each group.
[2] Figures in parentheses show number of ova recovered from ewes which came into estrus.

the ovaries of any of the nine ewes. Protruding follicles ranging from 3 to 7 mm. in diameter were present in the ovaries of these ewes. The presence of protruding follicles indicates that the ovaries were not in a state of complete quiescence.

None of the ewes in group II (those receiving only PMS) came into estrus within 10 days after the injection. During this period one of the ewes acted as though she were in estrus but would not stand when the ram attempted to mount. Upon examination at time of slaughter all had corpora lutea present on their ovaries. The number of corpora ranged from 1 to 3 and the average number was 1.8 per ewe (table 2).

Three of the nine ewes receiving one injection of progesterone (group III) came into estrus. Four ova were recovered from these ewes but none of them was fertilized. Estrus occurred from 1 to 2 days after the PMS injection. All the ewes but one had ovulated and this ewe had a large and slightly luteinized follicle 17 mm. in diameter present at the time of slaughter. This ewe did not show estrus.

In the groups receiving two and three injections of progesterone prior to PMS (group IV and group V), six of the nine ewes in each group came into estrus and all ewes in each group had ovulated. Three of eight ova recovered from ewes in group IV were fertilized and five of nine ova from group V were fertilized.

When four injections of progesterone were given prior to PMS (group VI), eight of nine ewes came into estrus, and in the group receiving five injections of progesterone prior to PMS (group VII), all of the ewes came into estrus. All of the ewes in these two groups had ovulated; 60.0 percent of the ova were fertilized in group VI and 53.3 percent in group VII.

When five injections of progesterone were given without the PMS (group VIII), four of nine ewes came into estrus and five had ovulated. All the ewes showing estrus had ovulated, and one which did not come into estrus had also ovulated. The ewes in this group did not come into estrus until 4 or 5 days after the last injection of progesterone. Five ova were recovered and three were fertilized.

The average over-all ovulation rate was 1.52 per ewe and the over-all fertility was 49.0 percent. Length of the induced estrus ranged from 1 to 3 checks with the average being 1.4 checks (table 3). Since checking for estrus was done at 24 hour intervals, the average duration of estrus was 33.6 hours.

Discussion

There was a definite relationship between the length of time during which the ewes were under progesterone treatment and the percentage showing synchronous estrus and ovulation. All ewes receiving PMS had ovulated except one, regardless of whether they had been previously injected with progesterone. None of the ewes came into estrus when PMS was injected without previous progesterone treatment. Thus, it appears that the problem is not one of inducing ovulation, but rather one of getting estrus to accompany ovulation or of inducing synchronous estrus and ovulation.

PMS alone did cause all of the treated ewes to ovulate, but none

of them came into heat (table 2, group II). This is in accord with previous reports on the use of this hormone. The simplest working hypothesis for the action of progesterone in inducing estrus when given prior to PMS is merely one of aligning the ovaries of all ewes so that when PMS is injected they are all at a common starting point in their inherent rhythmic pattern. Evidence in the literature (previously cited) shows that the ovaries are not completely quiescent or inactive in the ewe during anestrus and that a rhythmic pattern similar to that of the breeding season is evident. When PMS is injected alone, only a small percentage of ewes will usually respond with synchronous estrus and ovulation. It is possible that the ewes which respond with estrus and ovulation are those whose ovaries are

TABLE 3. AVERAGE LENGTH OF INDUCED ESTRUS

Group [1]	Number of Ewes Showing Estrus	Total Number of Estrual Checks for Ewes in Each Group [2]
III	3	4
IV	6	8
V	6	8
VI	8	12
VII	9	14
VIII	4	5
Total	36	51
Average	..	1.4

[1] None of the ewes in Groups I and II showed estrus.
[2] Checking for estrus was done once daily; therefore, on the average one check is equal to 24 hours duration.

naturally at a responsive stage in the rhythmic pattern or ewes whose ovaries have attained complete quiescence. The responsive stage may be defined as being analogous to the breeding season stage of a waning corpus luteum. Hammond, Jr., Hammond and Parkes (1942) have shown that estrus will occur if the ovulating stimulus is given in the presence of a waning corpus luteum. During anestrus if the ewes are all synchronized to a responsive stage of a subthreshold rhythmic pattern by progesterone, then PMS or any ovulating stimulus may result in synchronous estrus and ovulation.

The reproductive systems of the ewes which received progesterone for the longest period of time (group VII) may have been in a stage similar to that of ewes in which ovulation is induced by PMS and

followed 16 days later by a second injection which results in an increased percentage of ewes coming into estrus. The increasing percentage of ewes showing synchronous estrus and ovulation as a result of a longer period under progesterone treatment is difficult to explain on the basis of increased gonadotrophic output by the pituitary gland in view of the similar ovulation rates (table 2) among the different treatment groups.

Results from the group receiving progesterone alone (group VIII) are not in complete harmony with the hypothesis that progesterone did not result in additional output of gonadotrophic hormone by the pituitary gland. In this group five of the ewes ovulated and four of these came into estrus, which may appear to be evidence that progesterone had by some method, either directly or indirectly, stimulated the pituitary gland to additional output of gonadotrophic hormone. However, in the groups receiving progesterone prior to PMS there is no indication that progesterone did augment the action of PMS with regard to luteinizing action, since the ovulation rate for the group receiving PMS alone was 1.8 per ewe and this was higher than the ovulation rate for any of the groups. Gonadotrophic augmentation of PMS by progesterone through the pituitary gland would appear to furnish no evidence, however, for the occurrence of synchronous estrus and ovulation since different levels of PMS alone have not been found to increase the percentage of ewes which respond with estrus (Cole and Miller, 1935; Robinson, 1950). Thus it appears that the action of progesterone is not merely that of augmenting the effect of PMS.

In view of the longer interval (4 to 5 days) from the end of treatment to estrus in the group receiving only progesterone, it is possible that the occurrence of estrus and ovulation in some of these ewes may result from a "rebound effect." During progesterone treatment there may be damming up of small amounts of gonadotrophic hormone in the pituitary gland and, when progesterone therapy is ended, the output of gonadotrophic hormone by the pituitary gland is great enough in some of the ewes to cause ovulation. Estrus would accompany ovulation in these animals since synchronization of the ovaries had been effected by the progesterone treatment. The above explanation for the action of progesterone in anestrual ewes would follow more closely the present concept of the mechanism for the reciprocal relationship between the ovaries and the pituitary gland than does the explanation that progesterone acts as a stimulating

agent on the pituitary gland to cause increased output of gonadotrophic hormone.

The significance of the study reported here lies in the demonstration that progesterone, if given for a sufficient period of time prior to PMS injection, will synchronize the ovaries of anestrual ewes so that all treated ewes will respond with synchronous estrus and ovulation. In view of this finding, it is proposed that in ewes during the non-breeding season the ovaries pass through a transitional period from the breeding season to complete quiescence and from complete quiescence to the onset of the breeding season, during which time they follow a rhythmic pattern similar to that during the breeding season. It is further proposed that the period of complete quiescence is of variable duration and may not be attained at all in ewes of certain breeding or under certain environmental conditions. It is possible that failure of all but a low percentage of anestrual ewes treated only with gonadotrophic hormone to respond with synchronous estrus and ovulation may be due to treating during this transitional period. Synchronizing the ovaries in a group of anestrual ewes during the transitional period prior to treatment with a gonadotrophic hormone will result in a high percentage of treated ewes responding with synchronous estrus and ovulation.

Further study is necessary before the above explanation for the mechanism by which progesterone is effective in inducing synchronous estrus and ovulation in anestrual ewes can be completely accepted.

Summary

Anestrual ewes were injected with PMS alone, progesterone alone and progesterone for various periods followed by PMS in late May and June, and the incidence of estrus, ovulation, and fertilization was studied.

None of the control ewes came into estrus nor had any ovulated during the experimental period.

Injecting anestrual ewes with 500 IU of PMS alone resulted in ovulation but was not accompanied by estrus.

When progesterone was injected in approximately physiological amounts for a period of 15 days prior to PMS, synchronous estrus and ovulation occurred in all ewes.

Treatment with progesterone alone for a period of 15 days resulted in synchronous estrus and ovulation in four out of nine ewes.

Ova ovulated in anestrual ewes by PMS injection following progesterone treatment are fertilizable.

Estrus induced by this treatment was approximately of normal duration, i.e., the average duration in this study was 33.6 hours.

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VARIATION IN PENIS DEVELOPMENT IN RAM LAMBS

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AT birth the glans penis and processus urethra of the ram lamb are tightly adhered to the preputial mucosa with the processus urethra lying in a groove on the wall of the prepuce. As the lamb grows older, the adhesions gradually break down, beginning at the distal end and proceeding proximally. Thus the processus urethra becomes free first and, as the adhesions continue to break down, the prepuce can be everted and the penis exposed. According to Burrows (1949) the separation of the prepuce and the penis is due to the keratinization of the middle layers of cells of the balanopreputial fold.

Johnstone (1948) was apparently the first to publish a description of this phenomenon in lambs. He found that development was a gradual process that could be divided into several stages. His observations indicated little development before the lambs were 10 weeks old, gradual development from 10 to about 20 weeks, and fairly rapid development after that time. He did state, however, that development was not complete in all lambs at 5 months of age. Johnstone also reported that in a majority of adult wethers, the penis and related structures resembled those of very young ram lambs, indicating that the process of penis development was controlled by the testis hormone. He did note, however, that the rate of development appeared to be influenced to some extent by level of nutrition and general rate of growth.

Burrows (1949) states that the glans penis is adhered to the prepuce in young males of several species of laboratory animals (e.g., rabbit, cat). He reviews the work of a number of investigators which indicates rather clearly that the process of development is controlled by the testis hormone since the penis of an animal castrated early in life remains in an infantile condition, i.e. adhered to the prepuce, unless androgen is administered, in which case development proceeds as in the intact animal.

A few observations of penis development in ram lambs were made in connection with other studies (unpublished) at this Station in

[1] U. S. Sheep Experiment Station and Western Sheep Breeding Laboratory, Dubois, Idaho, in cooperation with the University of Idaho.

[2] The dienestrol used in this experiment was furnished by the Ortho Research Foundation, Raritan, New Jersey, through the courtesy of Dr. V. R. Berliner.

1939 and 1940. There appeared to be considerable variation in stage of development in ram lambs of about the same age. This study was undertaken to determine the amount of variation in penis development of ram lambs and to attempt to identify some of the causes of variation.

Materials and Methods

Earlier observations indicated that the process of penis development could be divided into five fairly well defined stages. These stages were assigned numerical scores as follows:

1=Infantile condition
2=Processus urethra free
3=Tip of glans penis free
4=Free to bottom of glans penis or slightly below
5=Adult condition

These stages of development are illustrated in figures 1–5.

The stage of penis development was scored at weaning time in 1250 ram lambs born in 1951. Data on 41 other ram lambs weaned were not used because of missing scores or uncertain parentage. The ages at scoring ranged from 91 to 155 days.

All sums of squares and cross products were calculated within breeds and type of mating (inbred line, cross line, etc.) and the net sums of squares and cross products were pooled to obtain average, within-group correlations (Snedecor, 1946).

Results and Discussion

Effect of Age

Although lambs with developmental scores of 3 and 5 were found at ages of 91 and 103 days respectively, the organs of a high percentage of lambs in the older age groups were still in an infantile condition at the time of examination (table 1). The youngest lamb examined (91 days old) was in stage 3 and the oldest lamb (155 days old) was in stage 1. Therefore neither the youngest age nor the oldest age at which penis development proceeds beyond stage 1 can be determined from these data.

Within breeds there was a decided tendency for the developmental scores to increase with age. The simple correlation coefficient (r) between age and penis score of 0.092 was highly significant (table 2). However, a comparison of the multiple correlation coefficient (R) of

TABLE 1. DISTRIBUTION OF PENIS SCORES OF RAM LAMBS BY AGES

Age	COLUMBIAS: Number of Lambs with a Penis Score of 1	2	3	4	5	Total	Mean Score	TARGHEES: Number of Lambs with a Penis Score of 1	2	3	4	5	Total	Mean Score
days														
<100	2	0	1	0	0	3	1.67	0	0	2	0	0	2	3.00
100–104	1	2	1	0	0	4	2.00	3	4	1	0	1	9	2.11
105–109	5	3	2	1	2	13	2.38	12	10	8	4	1	35	2.20
110–114	25	27	22	8	8	90	2.41	43	31	37	8	11	130	2.33
115–119	38	29	24	12	19	122	2.55	32	48	31	7	10	128	2.34
120–124	13	13	12	5	3	46	2.39	7	15	13	6	7	48	2.81
125–129	..	..	..	..	..	..	....	2	3	0	0	0	5	1.60
130–134	..	..	..	..	..	..	....	0	1	1	0	0	2	2.50
135–139	..	..	..	..	..	..	....	..	..	..	..	..	..	....
140 and over	..	..	..	..	..	..	....	..	..	..	..	..	..	....
Total	84	74	62	26	32	278	2.45	99	112	93	25	30	359	2.37

Age	RAMBOUILLETS: Number of Lambs with a Penis Score of 1	2	3	4	5	Total	Mean Score	TOTAL ALL BREEDS: Number of Lambs with a Penis Score of 1	2	3	4	5	Total	Mean Score
days														
<100	3	0	0	0	0	3	1.00	5	0	3	0	0	8	1.75
100–104	..	..	..	..	..	0	....	4	2	2	0	1	13	2.08
105–109	1	0	1	0	0	2	2.00	18	13	11	5	3	50	2.24
110–114	11	4	2	0	0	17	1.47	79	62	61	16	19	237	2.30
115–119	48	20	9	1	5	83	1.73	118	97	64	20	34	333	2.26
120–124	80	54	22	10	2	168	1.81	100	82	47	21	12	262	2.10
125–129	94	59	23	8	10	194	1.87	96	62	23	8	10	199	1.86
130–134	48	39	20	5	10	122	2.10	48	40	21	5	10	124	2.10
135–139	12	7	1	0	3	23	1.91	12	7	1	0	3	23	1.91
140 and over	1	0	0	0	0	1	1.00	1	0	0	0	0	1	1.00
Total	298	183	78	24	30	613	1.87	481	369	233	75	92	1250	2.14

age and weight with penis score (0.4403) and the simple correlation between weight and penis score (0.4401) indicates that the effect of age on penis development in this study was due almost entirely to the tendency for the older lambs to be heavier at scoring time.

Effect of Body Weight and Condition

The correlation coefficient of 0.440 between body weight and penis score was highly significant. Likewise the correlation of —0.270 between condition (degree of fatness) and penis score was also highly significant. This indicates that penis development proceeds more

TABLE 2. SIMPLE CORRELATION AND REGRESSION COEFFICIENTS BETWEEN VARIOUS WEANLING TRAITS OF RAM LAMBS [1]

Trait	Inbreeding	Body Weight	Condition	Staple Length	Index	Penis Score	
	r	*r*	*r*	*r*	*r*	*r*	*b*
Days growth	—0.00	0.18**	—0.17**	0.11**	0.02	0.09**	0.020
Inbreeding	....	—0.13**	0.08	0.01	0.04	—0.09*	0.019
Body weight	....	....	—0.70**	0.34**	0.56**	0.44**	0.040
Condition	....	....	....	—0.26**	—0.24**	—0.27**	0.017
Staple length	....	....	....	....	0.34**	0.17**	0.404
Index	....	....	....	....	....	0.31**	0.025

* Significant at the 5% level.
** Significant at the 1% level.
[1] Degrees of Freedom=1259 for all correlations except those involving inbreeding where d/f=706.

rapidly in the heavier, fatter lambs. This is in agreement with the report of Johnstone (1948) who did not present any data but did state that his observations indicated that penis development was affected by nutrition and general rate of growth.

Effect of Breed and Type of Mating

There were significant breed differences in development; 278 Columbias and 359 Targhees weaned at average ages of 115.2 and 114.7 days, respectively, had average developmental scores of 2.45 and 2.37, while 613 Rambouillets, weaned at an average age of 124.9 days, had an average score of only 1.87. The tests of significance were made on the unadjusted scores. The differences between the Rambouillets and the other two breeds would have been slightly greater if an adjustment for age had been made. On the other hand, adjustment for age would have reduced the difference between the Columbias and Targhees very slightly, but it is not likely that the

difference between these two breeds ($X^2=5.078$, $P<0.05$ but >0.02) could have been caused by a difference in average age of only one-half day. It seems more likely that the breed differences in penis scores were largely due to breed differences in body weight. The between-breed correlation between body weight and penis score was 0.981.

The Rambouillets were somewhat more variable in penis development than the other breeds as shown by the larger coefficient of variation. The coefficients of variation were higher in inbred animals than in outbreds in Rambouillets and Columbias but not in Targhees (table 3).

There was a significant negative correlation of —0.086 between inbreeding coefficient and penis score. It is likely that this correlation

TABLE 3. EFFECT OF BREED AND TYPE OF MATING ON PENIS DEVELOPMENT OF RAM LAMBS

Breed	Type of Mating	Number of Animals	Mean Age	Mean Penis Score	Std. Dev. Penis Score	Coeff. Var. Penis Score
			days			%
Columbia	Inbred lines	176	115.5	2.35	1.31	55.76
	Test pens	26	115.2	2.58	1.18	45.83
	Line crosses	76	114.5	2.64	1.34	50.93
	All	278	115.2	2.45	1.32	53.88
Targhee	Inbred lines	216	114.3	2.34	1.17	49.49
	Test pens	131	114.3	2.47	1.22	49.60
	Breed crosses	12	125.8	2.00	1.48	73.85
	All	359	114.7	2.37	1.20	50.65
Rambouillet	Inbred lines	346	125.1	1.82	1.11	61.08
	Test pens	85	125.2	1.87	1.03	54.90
	Line crosses	80	123.6	1.82	1.03	56.82
	Selected controls	102	125.2	2.04	1.08	53.13
	All	613	124.9	1.87	1.09	58.29

is largely a reflection of the depressing effect of inbreeding on body weight since when weight was held constant, the partial correlation between inbreeding and penis score was only —0.032.

Effect of Staple Length and Weanling Index

The correlations of staple length and of weanling index with penis score were 0.175 and 0.308, respectively. Both were significant but it appears that their association with penis score arises from their association with body weight, since the multiple correlations of either of them and body weight with penis score were only very slightly larger than the simple correlation between body weight and penis score.

Effect of Age of Dam and Type of Birth and Rearing

Lambs born and raised as singles and lambs born as twins but raised as singles had average penis scores of 2.30 and 2.32, respectively, while lambs born and raised as twins had an average score of only 1.88 (table 4). These highly significant differences are probably due to the lighter weaning weight of the lambs weaned as twins.

Lambs out of ewes 2 and 3 years old had average penis scores of 2.18 and 2.21, respectively. Lambs out of ewes 4 to 7 years old had

TABLE 4. EFFECT OF AGE OF DAM AND TYPE OF BIRTH AND REARING ON PENIS DEVELOPMENT IN RAM LAMBS

Age of Dam	Item	Type of Birth and Rearing: Born and Weaned as Singles	Born as Twins Weaned as Singles	Born and Weaned as Twins	Born as Triplets Weaned as Singles	Born as Triplets Weaned as Twins	All
years							
2	No. of lambs	204	15	10	0	0	230
	Mean penis score	2.16	2.60	1.90	....	....	2.18
3	No. of lambs	161	33	81	0	0	275
	Mean penis score	2.39	2.15	1.88	....	....	2.21
4	No. of lambs	69	35	106	1	2	213
	Mean penis score	2.17	2.57	2.02	2.00	1.50	2.15
5	No. of lambs	66	34	111	0	3	214
	Mean penis score	2.35	2.56	1.94	....	2.67	2.17
6	No. of lambs	46	28	95	0	2	171
	Mean penis score	2.70	1.96	1.73	....	2.00	2.03
7	No. of lambs	27	23	46	0	0	96
	Mean penis score	2.67	2.39	1.70	....	....	2.14
8	No. of lambs	14	5	23	0	0	42
	Mean penis score	1.93	1.40	1.87	....	....	1.83
9	No. of lambs	5	4	0	0	0	9
	Mean penis score	2.00	1.75	....	....	....	1.89
All	No. of lambs	593	177	472	1	7	1250
	Mean penis score	2.30	2.32	1.88	2.00	2.14	2.14

X^2 for age of dam=31.85 $P<0.01$.
X^2 for type of birth=54.85 $P<0.01$.

average penis scores ranging from 2.03 to 2.17. The scores of lambs out of 8- and 9-year-old ewes were only 1.83 and 1.89, respectively. A much lower proportion of lambs were born and raised as twins from ewes 2 and 3 years old than from ewes 4 to 7 years old. This would tend to decrease the average penis scores of lambs from the latter group since twin lambs had lower scores. The lower penis scores of lambs out of 8- and 9-year-old ewes were probably associated with the lighter weaning weights of these lambs.

Heritability of Penis Score

Estimates of heritability of penis development of ram lambs were obtained from half-sib, intraclass correlations. Estimates were not

obtained for line-crosses and breed-crosses because of uncertainty concerning the correction for inbreeding and because these groups were generally small. The estimates of heritability were quite variable among the breeds and types of mating and the standard errors were quite large (table 5). It is emphasized that these estimates are based on a limited amount of data and that conclusions regarding breed or type of mating differences in heritability should remain tentative until data from additional years have been obtained.

Hormonal Control of Penis Development

In 1952 an experiment was conducted to secure evidence on whether or not the process of penis development in ram lambs is controlled

TABLE 5. HERITABILITY OF PENIS DEVELOPMENT OF RAM LAMBS AS ESTIMATED BY HALF-SIB CORRELATIONS

Breed	Type of Mating	Degrees of Freedom		Estimate of Heritability	Standard Error [1]
		between Sires	within Sires		
Columbia	Inbred lines	14	161	0.110	0.099
	Test pens	2	23	—.344	0.389
Targhee	Inbred lines	16	199	0.008	0.059
	Test pens	17	113	0.240	0.244
Rambouillet	Inbred lines	40	305	0.356	0.103
	Test pens	14	19	—.452	0.758
	Controls	5	96	0.704	0.325

[1] A modification of the standard error of the intraclass correlation obtained by the method of Fisher (1948).

by the testis hormone. Seventy Rambouillet or Columbia-Rambouillet ram lambs that had penis scores of 1 at weaning time either 1 or 2 weeks earlier were divided at random into seven lots. They received various treatments as indicated in table 6. Ten other lambs that were in stage 3 the day the experiment began were divided at random into two lots (lots 8 and 9, table 6). The testosterone propionate was dissolved in alcohol and injected subcutaneously. The aqueous suspension of dienestrol was also injected subcutaneously. Injections of both hormones were made once a week for 3 weeks and the last observations on penis development were made 2 weeks after the last injection. The castrations were performed with Burdizzo forceps and

at the end of the experiment all lambs were carefully examined for testicular atrophy. Three lambs that did not exhibit pronounced atrophy of both testes were judged not to have been castrated and data on them were not included in the analysis of the results. This accounts for the discrepancy between the number of animals started on experiment and the number shown in table 6.

Penis development was arrested by castration in lambs in the early stages of development and also in lambs in a later stage of development. In both cases the differences between the castrated lambs and their controls were significant. The mean change in penis score in

TABLE 6. EFFECT OF SEX HORMONES AND CASTRATION ON PENIS DEVELOPMENT IN RAM LAMBS

Lot	No. of Animals	Treatment	Mean Initial Penis Score	Mean Final Penis Score	Mean Change
1	10	Control	1.30	2.80	1.50
2	10	5 mg. TP [1]	1.60	3.40	1.80
3	10	10 mg. TP [1]	1.40	3.00	1.60
4	10	20 mg. TP [1]	1.20	3.10	1.90
5	10	12.5 mg. Est.[2]	1.10	1.30	0.20*
6	9	Castrated	1.22	1.44	0.22*
7	9	Cast. 10 mg. TP [1]	1.11	1.55	0.44
8	5	Control	3.00	5.00	2.00
9	4	Castrated	3.00	3.25	0.25*

* Change is significantly less ($P<0.05$) than that of corresponding control lot.
[1] Testosterone propionate.
[2] Dienestrol.

the lot that received weekly injections of 10 mg. of testosterone propionate was not significantly different from the change in the intact-control lot, but neither was it significantly different from the change in the castrated lot. Therefore, further experimentation will be necessary to establish conclusively whether or not the effect of castration on penis development can be overcome by the administration of testosterone propionate. However, several investigators (see Burrows, 1949) have reported that separation of the penis and prepuce takes place in castrated animals of other species when androgens are administered. Development of the penis was also arrested by weekly injections of 12.5 mg. of the synthetic estrogen, dienestrol. These results indicate that the presence of the testis hormone is necessary for penis develop-

Figure 1. Stage 1—Infantile condition. Processus urethra and glans penis adhered to prepuce.

Figure 2. Stage 2—Processus urethra free. Remainder of penis adhered to prepuce.

ment and also indicate that estrogen will arrest development in intact animals.

The results of administering testosterone propionate to normal animals were inconclusive. Since none of the lots receiving testosterone propionate developed significantly faster than the controls, one might conclude that a threshold phenomenon is involved, and that development cannot be accelerated by additional hormone. It is entirely possible, however, that development of the penis would be accelerated by the administration of exogenous male sex hormone with higher

Figure 3. Stage 3—Tip of glans penis free.

Figure 4. Stage 4—Free of adhesions to bottom of glans penis.

Figure 5. Stage 5—Adult condition. Processus urethra, glans penis and body of penis free of adhesions.

dosages, more frequent injections and/or other changes in the experimental procedure. Further experimentation will be necessary to clarify this.

Discussion

More work will be necessary before the significance of the findings reported here can be evaluated. It would appear especially important to study the relationship between penis development in lambs at a fairly young age (e.g., weaning time) and subsequent reproductive performance as measured by semen production and fertility in actual breeding. At the present time very little can be done in the way of selecting for high fertility in sheep because practically all selections must be made before information on fertility becomes available. Any reasonably accurate measure of potential reproductive performance that is easily obtainable and available early in the life of the animal would be a valuable aid in selecting for high fertility.

Additional data are needed to obtain more reliable estimates of the heritability of penis development. Further investigations to determine more precisely the role of the testis hormone in penis development would also appear to be worthwhile.

Summary

At birth the glans penis and processus urethra of the ram lamb are adhered to the preputial mucosa, the urethral process lying in a groove

on the wall of the prepuce. The adhesions gradually break down as the lamb grows older so that the prepuce can be everted and the penis exposed. The developmental process can be divided in five fairly well defined stages which were scored as follows: (1) infantile condition, (2) urethral process free, (3) tip of glans penis free, (4) free to bottom of glans penis or slightly below and (5) adult condition.

The stage of development was scored at weaning time, at ages of 91 to 155 days, in 1250 ram lambs born in 1951. Although developmental scores of 3 and 5 were found in lambs 91 and 103 days old respectively, the organs of a number of the oldest lambs examined were still in an infantile condition.

There were significant breed differences in development; 278 Columbias and 359 Targhees, both weaned at an average age of 115 days, had mean scores of 2.45 and 2.37, respectively, while 613 Rambouillets, weaned at an average age of 125 days, had a mean score of only 1.87. Corresponding standard deviations for the three breeds were 1.32, 1.20 and 1.09. The coefficients of variation were higher in inbreds than in outbreds of the same breed in Columbias and Rambouillets but not in Targhees.

Simple correlation coefficients of penis scores with other weanling traits were: age, 0.092**; inbreeding, —0.086*; weight, 0.440**; condition, —0.270**; staple length, 0.175**; and index, 0.308**.

Lambs born and raised as singles and lambs born as twins but raised as singles had average penis scores of 2.30 and 2.32, respectively, while lambs born and raised as twins had an average score of 1.88.

Lambs out of ewes 4 to 7 years old had slightly lower average scores than lambs out of 2- and 3-year-old ewes probably because a higher proportion of them were twins. Lambs from 8- and 9-year-old ewes had much lower scores than lambs from younger ewes.

Heritability estimates obtained from half-sib correlations were quite variable for the various breeding groups. The estimate for Rambouillets was considerably higher than the estimate for Columbias and Targhees.

An experiment conducted in 1952 indicates that the developmental process is under the control of the testis hormone. Development was arrested by castration and by the administration of the synthetic estrogenic hormone, dienestrol. Results of administering testosterone propionate to intact animals were inconclusive.

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INFLUENCE OF A HIGH SALT INTAKE ON CELLULOSE DIGESTION

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COMMON salt is being used by stockmen in high concentration in range supplemental feeds to control the consumption of the supplemental feed by the individual animal. The amount of supplement eaten is governed by the salt content, and the consumption can be elevated or lowered by varying the amount added.

This practice has proved highly successful. Supplemental feed can be made readily available to cattle scattered over extensive range areas. The use of salt reduces labor cost because several days' supply of the supplement can be placed in a self-feeder. This practice also prevents a few animals from eating too much and insures that all animals will have an equal access to the supplement.

Research work completed to date by Cardon *et al.* (1951), Pistor *et al.* (1950), Savage and McIlvain (1951) and Weir and Miller (1953) has shown that if normal precautions are taken salt used in this way is not harmful to the animal. It is conceivable, however, that increasing the salt concentration in the rumen fluid may have a detrimental effect on rumen microorganisms even though salt may not have any direct toxic effect on the animal. The ingestion of 1 pound of salt by an animal of average size might increase the salt concentration of the rumen fluid by 0.3 to 0.5 percent, depending on the amount of fluid present. As cellulose in the plant is an important source of the energy available to the animal, and as its decomposition is dependent entirely upon the activity of certain rumen microorganisms, an investigation was made of the effect on cellulose digestion as well as on digestible energy of an increased concentration of salt in the rumen fluid.

Experimental

In Vivo Experiments

Three 1000 pound Hereford cows were placed on a daily ration of 15 pounds ground alfalfa hay. Representative feed and feces samples were collected after a preliminary 2 weeks' feeding period. Feces

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samples were collected daily at 1:00 p.m. for 5 consecutive days. Results reported are the average values of the five samples collected. Digestible cellulose and digestible gross energy were calculated by the lignin ratio method. Nitrogen was determined by the Kjeldahl method, cellulose determined by the method of Matrone *et al.* (1946), and lignin by the method of Ellis *et al.* (1946). Heat of combustion was determined using the Parr oxygen bomb.

In the next trial 1.8 pounds of salt were added to the daily ration after the digestible cellulose and gross energy had been determined. The salt was mixed in the ground alfalfa hay and did not materially influence its consumption. Digestibility determinations were made on the high salt ration after a two-week preliminary adjustment period.

In the last trial two pounds of salt per day were given to the animals. This salt was given as a drench, 1 pound in the morning

TABLE 1. INFLUENCE OF A HIGH SALT INTAKE ON THE DIGESTIBILITY OF ALFALFA HAY

Item	Composition	Percent Digestibility		
		Without Added NaCl	1.8 Lb. NaCl/Day in Ration	2 Lb. NaCl/Day as Drench
Protein	15.7%	75.5	72.3	74.1
Cellulose	29.9%	55.0	55.1	56.5
Crude energy	4.31 Cal./gm.	61.4	61.2	61.0

and 1 pound in the evening. Digestibility determinations were again made on this ration after a two-week preliminary adjustment period. There was a slight scouring for the first 2 days after the animals were placed on this ration; thereafter, the salt had no apparent detrimental effect on the animals.

In Vitro *Experiments*

Because of absorption from the rumen, the salt concentration may change rapidly in the rumen fluid. Consequently, an *in vitro* method, in which the salt concentration could be kept constant, also was used to measure changes in digestibility of cellulose.

The "artificial" rumen consisted of a battery of four 1-liter flasks placed in a constant temperature bath which was maintained at 39° C. Rumen fluid plus the alfalfa to be fermented was placed in the flasks. The initial contents of the flasks are shown in table 2. Each flask

was fitted with a two-hole stopper; one hole served as an inlet for nitrogen gas, the other as an outlet for waste gases and nitrogen. The flasks were connected in series. The glass tube inlet penetrated the liquor in the flask so that bubbles were formed as the gas swept through. The flow of the gas varied from 80 to 100 bubbles per minute.

The rumen fluid used was drawn from a mature cow through a fistula. The fluid was stored in a dark brown bottle at body temperature and was used within an hour after collection. Just before using,

TABLE 2. *IN VITRO* DECOMPOSITION OF CELLULOSE BY RUMEN FLUID

Contents of Flask	pH at Finish of Experiment [1]	Percent of Cellulose Digested
17 gm. alfalfa 600 ml. rumen fluid	5.5	37.4
17 gm. alfalfa 600 ml. rumen fluid 2.4 gm. NaCl	5.4	36.0
17 gm. alfalfa 600 ml. rumen fluid 20 ml. 1-molar phosphate buffer (pH 7.1)	5.6	38.0
17 gm. alfalfa 600 ml. rumen fluid 20 ml. 1-molar phosphate buffer (pH 7.1) 2.4 gm. NaCl	5.6	36.6

[1] Initial pH=6.8.

the rumen liquor was filtered through glass wool to remove any solid particles.

Four fermentation trials were made. Controls consisting of rumen fluid and alfalfa were included with each fermentation. This was necessary because of the variation in composition and activity of rumen fluid. Checks were also made on the influence of salt and phosphate buffer on the pH after fermentation. After assembly with the desired constituents, the flasks were incubated for 12 hours. All flasks were swirled by hand every hour for the first 10 hours of the experiment to insure that all particles of alfalfa be exposed to the action of the rumen fluid. At the end of incubation, material in

the flask was filtered through a No. 40 filtering cloth, washed with water to remove all soluble substances, dried at 80° C. for 24 hours, weighed, then reground to 40-mesh and analyzed.

Results

Results of the *in vivo* digestion experiments are shown in table 1. Digestible protein, cellulose, and gross energy were not appreciably altered when the salt was added to the diet.

The results from one artificial rumen digestion trial are shown in table 2. These results are typical of all trials.

It can be seen that there was only a slight difference in the percentage of cellulose digested in each flask. The addition of sodium chloride or 3.3 percent molar phosphate buffer (pH=7.1) had no effect on the final pH after fermentation. Results show that increasing the salt concentration (by adding 2.4 gm. NaCl per 600 ml. rumen fluid) had no appreciable effect on the activity of those rumen microorganisms responsible for cellulose digestion, as measured by changes in digestibility.

Discussion

Stockmen in the Southwest use an amount of salt which will limit the consumption of supplement by range animals to from 1 to 3 pounds daily. This means that the animals may consume up to 1 pound of salt each day. The results reported here show that cattle may eat up to 2 pounds of salt daily without suppressing the digestibility of cellulose or gross energy.

The amount of cellulose digested in the *in vitro* experiment does not compare with the 90 percent digested as reported by Burroughs *et al.* (1950) or Louw *et al.* (1949). Alfalfa was the cellulose source used in this experiment. Cellulose in alfalfa is surrounded by natural protective barriers, such as lignin, which prevent the bacteria from obtaining complete access to the cellulose fraction. Previous experiments *in vivo* have shown that the cellulose in this alfalfa was only 51 percent digestible. A short incubation period was also used because it was felt that longer incubation under artificial conditions would tend to "enrich" types of microorganisms which were not normally active in the rumen.

These results show that the activity of the rumen microorganisms responsible for cellulose digestion is not decreased by the increased

salt concentration. Apparently the increase in osmotic pressure does not inhibit their metabolic activity to the point where there is interference with cellulose fermentation.

Summary

Digestion studies with cattle and artificial rumen fermentation experiments clearly show that the increased salt concentration of the rumen caused by feeding high salt rations did not cause a decrease in rumen microbial activity. Both types of experiments show that digestible cellulose as well as digestible gross energy is not altered by the increased consumption of salt.

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THE ROLE OF PLANT PIGMENTS IN DIGESTION TRIAL STUDIES

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Reid *et al.* (1950) first used natural occurring plant pigments as a marker in digestion trial work. In their studies the acetone extract of hays and feces produced absorption curves which invariably crossed one another at 406 mμ. They assumed that no changes occurred at this isobestic point and satisfactorily demonstrated its applicability as a marker in digestion trials under the experimental conditions imposed. In a later paper Reid *et al.* (1952) related the chromogen concentration of the dry matter of the feces to the percent of dry matter digested, thus providing a technique for studying forage digestion under grazing conditions. Kane *et al.* (1951) in a study of digestion trial techniques concluded that satisfactory results could be obtained in orchard grass studies using chromium oxide and plant pigments whereas lignin, total methoxy and lignin methoxy were unsatisfactory. Cook and Harris (1951) reported that the chromogen technique as a method of studying digestibility was unsatisfactory for sheep on desert range plants.

The applicability of plant pigments as a digestion trial marker depends upon their physical and chemical characteristics. Such pigments can thus be grouped into three classes: carotenoids, xanthophylls and chlorophylls. The carotenoids could not be used as a marker because of their instability and because they are absorbed to some extent during the digestive process. The chemical nature of carotenoids (Karrer and Jucker, 1950) reveals their inadequacies as a possible digestion trial marker. Xanthophylls are even less suitable. They are very sensitive to oxygen and acids, although 75 to 85 percent of the xanthophyll intake can be recovered in feces (Rogozinski, 1938).

It has been definitely established that chlorophylls are degraded in the intestinal tract of humans and animals. Inman and Rothemund

Paper No. A393, Contribution No. 2409 of the Maryland Agricultural Experiment Station, Dept. of Dairy Husbandry.

[1] The experimental data in this paper are taken, in part, from a thesis to be submitted by H. M. Irvin in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Dairy Husbandry, University of Maryland.

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(1932) reported that the major changes in chlorophyll occurred in the third stomach of the ruminant. Kortschagin (1924) stated that gastric juice would change chlorophyll into the first of its degradation products, the magnesium-free pheophytin. Rothemund and Inman (1932) reported the presence of phylloerythrin, pyrroporphyrin, rhodoporphyrin and phylloporphyrin in the third and fourth stomachs of the cow. Phylloerythrin is a further degradation product of pheophytin and is found in the digestive tract of ruminants and rodents according to Quin *et al.* (1935). These authors also pointed out that phylloerythrin is absorbed into the blood stream as indicated by use of a bile fistula. The phylloerythrin excretion was proportional to the chlorophyll intake. Fischer and Hendschel (1931) reported the presence of phylloerythrin in the intestinal canal of the ruminant. Fischer and Stadler (1936) showed the presence of pheophorbides in sheep feces. For a more complete summary of chlorophyll degradation products, the reader is referred to Wilstätter and Stoll (1928), Rothemund (1944), and Mayer and Cook (1943).

It is apparent from the review of literature that no pigments pass through the intestine totally unchanged. Although Rogozinski (1938) stated that 15 to 25 percent of ingested chlorophyll disappeared in the intestinal tract of sheep, such a disappearance might be due to the conversion of chlorophyll to the degradation products heretofore mentioned. No literature was observed concerning the quantitative absorption of the degradation products of chlorophyll and from the data included herein, such absorption is probably low. The fact that porphyrins are excreted by the bile and kidney (Dobriner and Rhoads, 1940; Roets, 1941; With, 1949) are additional factors which should be considered in studying feces pigments.

Experimental Procedure

This experiment was designed to study the pigments found in feed and feces. It was hoped that such a study might bring to light a pigment or pigments of such stability that they could be used as digestion trial markers.

Two lactating Jersey cows, 647 and 699, were placed on an all-alfalfa hay diet in December, 1951, and after a 10-day preliminary period, total collection of feces was made for a 5-day period. The digestion trial data are given in table 1.

Two hay and three feces samples were extracted for studying the pigments present. A 300 gm. air dry sample of No. 2 field-cured

alfalfa hay, after grinding through a 40 mesh screen in a Wiley Mill, was extracted with absolute ethyl alcohol in a ball mill overnight. The extracted pigments were separated from their residue by a large amount of alcohol washing on a fritted glass funnel. This washing was continued until the filtrate was clear. The alcoholic extract was diluted to 80 percent alcohol with 4 percent NaCl solution. Total volume at this point was slightly over 4 liters. A large separatory funnel was used for extracting the 80 percent ethyl alcohol with 300 to 500 ml. portions of petroleum ether (Skellysolve-F or SK-F). After vigorous shaking and then settling, the SK-F was drawn off with certain pigments extracted. This process was repeated six times, after which the color of the SK-F was only slightly yellow. The SK-F extracts were placed in one large suction flask and evaporated to almost dryness. Then the remaining solution was transferred to a 250 ml. volumetric and made to volume with benzene. This portion is

TABLE 1. DIGESTION TRIAL DATA

	No. 2 Alfalfa Hay Dry Matter Intake/Day	Average Daily Excretion of Dry Matter	Dry Matter Digestion Coeff.
	Kgm.	Kgm.	%
Cow 647	6.492	2.795	56.95
Cow 699	6.492	2.951	54.54

designated the "80 percent ethyl alcohol extract." The 80 percent alcohol itself was converted to 70 percent alcohol and the above extraction procedure repeated. Then the 70 percent alcohol was converted into 50 percent and then into 33⅓ percent with a repetition of the above procedures in each case. From each (SK-F)-benzene fraction an aliquot of 10 or 20 ml. was pipetted in a small suction flask and reduced to dryness. The residue was immediately dissolved in a small amount of benzene and the solution was transferred to a chromatographic column. The absorbents used were principally powdered sugar (sucrose) or dicalcium phosphate. If pigments tended to move very slowly on a dicalcium phosphate column, then a switch to sugar was made. If pigments tended to move down the column en masse or too rapidly, then dicalcium phosphate and sometimes $MgCO_3$ was used. The eluents consisted principally of SK-F, (SK-F)-benzene mixtures and small percentages of acetone or ethyl alcohol in SK-F or the above mixture. Here again the movement of the pig-

ments and their separation determined the type of eluent used. Pigments in most cases separated out individually although some mixing inevitably occurred. The above pigments after chromatographing were reduced to dryness *in vacuo* and then dissolved in a definite volume of benzene. Each pigment volume was corrected to a suitable density for reading on the Beckman DU Spectrophotometer, and pigment density curves were ascertained in the visible spectral region 320 to

TABLE 2. CHARACTERISTICS OF PIGMENTS ISOLATED IN ALFALFA HAY [1]

Chromatographic			Spectrophotometric Characteristics [3]					
			Wavelength			Pigment Concentration Units/gm./ml.		
Color on Column	Sample No.	Eluent	Max.	Min.	Max.	Max.	Min.	Max.
			mμ	mμ	mμ			
		80% EtOH fraction [2]						
Yellow	1	C_6H_6	415.0	446.0	459.0	1.48	0.72	0.79
Green	2	C_6H_6	415.0	.....	.....	47.01	....	....
Yellow	3	C_6H_6	415.0	434.0	437.0	1.09	0.55	0.56
Yellow	4	C_6H_6	416.0	474.0	490.0	4.88	0.83	0.95
Yellow	5	C_6H_6	415.0	475.0	485.0	2.51	0.38	0.41
Green	6	C_6H_6+0.3% EtOH	410.5	.....	.....	0.58	....	....
Green	7	C_6H_6+0.3% EtOH	406.0	.....	.....	2.22	....	....
Yellow	8	C_6H_6+0.5% EtOH	411.0	450.0	454.0	0.44	0.25	0.25
Green	9	C_6H_6+0.7% EtOH	430.0	437.0	457.0	0.50	0.49	1.03
Green	10	C_6H_6+0.7% EtOH	424.0	437.0	456.0	1.58	1.33	2.65
Green	12	C_6H_6+4.0% EtOH	411.5	.....	.....	1.34	....	....
		70% EtOH fraction [2]						
		SK—F/C_6H_6(1:1)						
Grey-Yellow	1	+0.2% EtOH	414.5	429.0	439.0	6.26	4.07	5.84
Yellow	2	+0.2% EtOH	414.5	428.0	438.0	1.99	1.47	1.95
Yellow	3	+0.2% EtOH	430.0	435.0	455.0	1.97	....	2.77
			.....	475.0	485.0	....	1.79	2.06
Yellow	4	+0.2% EtOH	408.0	423.0	.....	0.49	0.42	....
			433.0	444.0	.....	0.44	0.40	....
			456.0	474.5	486.0	0.42	0.25	0.28
Green	5	+0.5% EtOH	430.0	437.0	455.5	2.42	2.33	4.74
Yellow	6	+2.0% EtOH	420.0	431.0	446.0	0.38	0.34	0.49
Yellow	7	+2.0% EtOH	419.5	428.5	445.5	1.02	0.97	1.17
		50% EtOH fraction [2]						
		SK—F/C_6H_6(4:1)						
Yellow	1	+0.05% EtOH	415.0	431.0	439.5	1.40	0.92	1.30
Yellow	2	+0.2% EtOH	416.0	426.0	439.0	0.60	0.55	0.88
Yellow-Orange	3	+0.05% EtOH	433.5	440.5	456.0	3.26	3.23	4.07
			.....	475.0	486.0	....	2.84	3.39
Yellow	4	+0.5% EtOH	435.0	440.0	456.0	0.48	....	0.56
			.....	475.0	485.0	....	0.38	0.45
Yellow	6	+2.0% EtOH	410.5	.....	.....	0.33	....	....

[1] All samples were read spectrophotometrically in C_6H_6 (redistilled).
[2] $CaHPO_4 \cdot 2H_2O$, Baker's Analyzed, was used as the absorbent.
[3] The max.-min.-max. pigment concentrations were determined at the respective max.-min.-max. wavelengths as indicated for each sample.

500 mμ. In this manner a rather large number of pigments were isolated although some overlapping did occur.

With this technique a 300 gm. wet feces sample from a five-day composite sample was studied from cow 699. Six months later in

TABLE 3. CHARACTERISTICS OF PIGMENTS ISOLATED IN (ALFALFA HAY) FECES, COW 699 [1]

Chromatographic			Spectrophotometric Characteristics [4]					
			Wavelength			Pigment Concentration Units/gm./ml.		
Color on Column	Sample No.	Eluent	Max.	Min.	Max.	Max.	Min.	Max.
			mμ	mμ	mμ			
		80% EtOH fraction [2]						
Yellow	1	SK—F/C_6H_6(1:1)	462.0	482.5	484.0	1.48	1.19	1.20
Yellow	2	SK—F/C_6H_6(1:3)	414.0	.....	.....	4.40	....	....
Grey	3	SK—F/C_6H_6(1:4)	414.0	.....	.....	41.93	....	....
Yellow	4	SK—F/C_6H_6(1:4)	418.0	422.5	439.0	6.69	6.52	16.27
Yellow	6	SK—F/C_6H_6(1:1) +0.5% EtOH	414.0	.....	.....	4.04	....	....
Yellow	7	C_6H_6	416.0	472.0	484.5	2.82	0.66	0.76
Yellow-Green	8	SK—F/C_6H_6(1:1) +1.0% EtOH	409.0	442.5	455.0	5.73	3.51	4.12
		70% EtOH fraction [3]						
Yellow	1	SK—F	440.0	472.0	485.0	4.63	1.92	2.32
Yellow	2	SK—F/C_6H_6(1:1)	440.5	445.0	452.0	1.74	1.71	1.77
			.....	472.5	485.0	....	1.13	1.34
Yellow	3	SK—F/C_6H_6(1:1)	418.0	446.5	451.0	1.18	0.98	0.99
			.....	472.0	483.0	....	0.65	0.72
Green	4	SK—F/C_6H_6(1:1) +0.2% EtOH	431.0	437.0	458.0	0.59	0.58	1.27
Green	6	SK—F/C_6H_6(1:1) +1.0% EtOH	406.5	.....	.....	1.07	....	....
		50% EtOH fraction [3]						
Yellow	1	SK—F	430.0	435.0	456.0	2.36	....	3.28
			.....	474.0	485.0	....	2.39	2.87
Yellow	2	SK—F/C_6H_6(1:1)	430.0	435.0	455.5	1.27	....	1.72
			.....	474.0	485.0	....	1.23	1.47
Yellow	3	SK—F/C_6H_6(1:1)	430.0	435.0	452.5	0.94	....	1.16
			.....	471.0	482.0	....	0.84	0.94
Green	5	SK—F/C_6H_6(1:1) +0.5% EtOH	410.0	422.5	433.0	1.28	1.16	1.32
Green	6	SK—F/C_6H_6(1:1) +1.0% EtOH	413.5	435.0	444.0	0.63	0.48	0.51
		33⅓% EtOH fraction [3]						
Yellow	3	SK—F/C_6H_6(1:1) +0.1% EtOH	408.5	414.0	431.0	0.28	0.28	0.40
			.....	443.0	457.0	....	0.32	0.40
Yellow	5	SK—F/C_6H_6(1:1) +1.0%—8.0% EtOH	411.0	.....	.....	1.01	....	....
Yellow	6	EtOH	419.0	.....	.....	0.82	....	....

[1] All samples were read spectrophotometrically in C_6H_6 (redistilled).
[2] $CaHPO_4 \cdot 2H_2O$, Baker's Analyzed, was used as the absorbent.
[3] Powdered sucrose was used as the absorbent.
[4] The max.-min.-max. pigment concentrations were determined at the respective max.-min.-max. wavelengths as indicated for each sample.

June, 1952 the above procedures were repeated on a 50 gm. hay sample and on a 150 gm. feces sample (fifth collection day) from cow 699. A composite 100 gm. wet feces sample from cow 647 was studied beginning in July, 1952.

The number and types of pigments involved can be seen in tables 2 and 3. Table 2 includes the pigments isolated in the first hay sample studied and table 3 includes the pigments contained in the feces sample from cow 699 (five-day composite). The maximum and minimum absorption peaks and troughs are shown with their respective concentration expressed in units/gm. of dry matter/ml. of solution. These two tables also give the chromatographic characteristics of the pigments eluted, such as their color on the column, the eluent and absorbent used, the solvent in which the pigment was studied, and their relative order on the column as indicated by the sample number (the lowest number leaving the column first).

The concentration of the pigments is expressed in the following manner. The density reading of each wavelength recorded was standardized in the hay pigments by converting density, as read, to density per gm. of dry hay extracted per ml. of solvent (as read in the spectrophotometer). The corrected density is expressed as "units/gm./ml." In the case of the feces pigments, another factor is involved; namely, the undigestibility of the dry matter. The units/gm. of dry feces/ml. of solvent is multiplied by a fraction representing the undigestible portion of the dry matter intake. The product obtained can thus be compared directly with the "corrected" density readings of the hay.

Results

From the data given in tables 2 and 3, it is apparent that no one pigment exists in hay which passes through the intestinal tract unchanged. From the data and from the literature reviewed, no single pigment can be used as a marker in digestion trial studies. The one pigment which showed the most promise was pigment No. 2 of the 80 percent EtOH hay fraction and pigment No. 3 of the 80 percent EtOH feces fraction. This pigment with its maximum absorption at 415 mμ behaves spectrally and chromatographically like "pheophytin," the first degradation product of chlorophyll. It occurs in the hay probably from the technique used in separating the pigments. For example, the $CaHPO_4 \cdot 2H_2O$ used as an absorbent was slightly acid in solution, and chlorophyll is extremely sensitive to acids. In the feces, "pheophytin" results from the action of gastric juice

on chlorophyll (Kortschagin, 1924). The quantity of "pheophytin" observed is far in excess of any other pigments in the hay or feces.

When a composite curve is made of the total hay and total feces pigments, "pheophytin" controls very largely the characteristics of each curve. Figure 1 represents a composite curve of the hay pigments

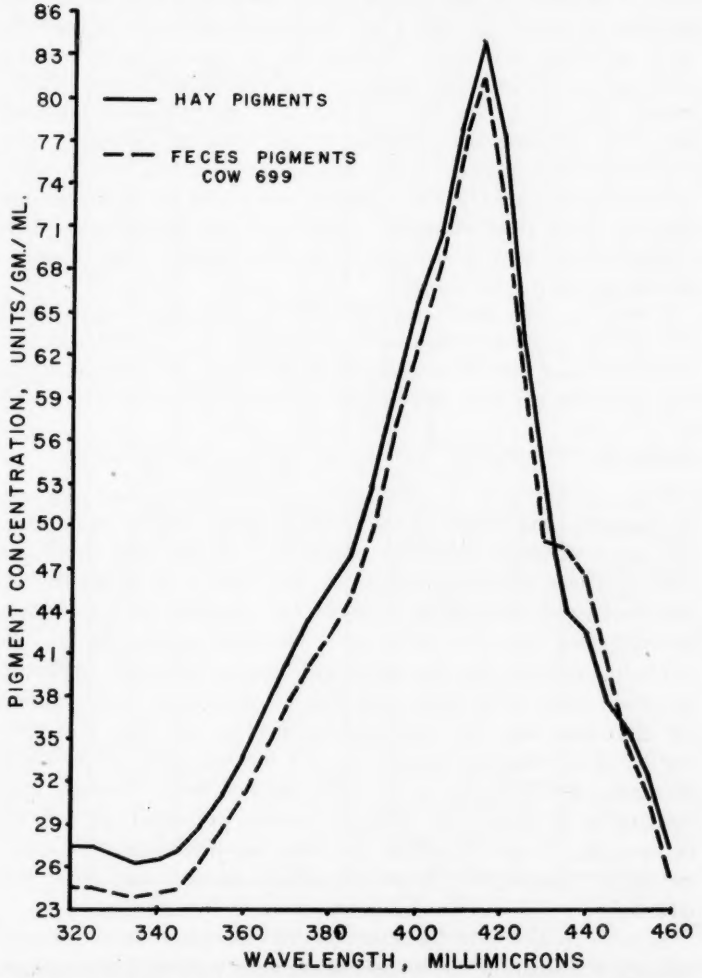

Figure 1. Composite spectrophotometric readings of pigments extracted from equivalent quantities of hay and feces.

recorded in table 2 and a similar curve of the feces pigments as given in table 3. A close similarity exists between the two curves and the spectral reading made at 415 mμ would indicate a 97.5 percent recovery of total pigments. This recovery appears remarkable in view of the variety of pigments involved, although "pheophytin" is the major constituent in both curves. When the alfalfa hay sample was processed as above, in June, 1952, and a feces sample from cow 699 (fifth collection day only) extracted in the same month, a composite picture of individual pigments chromatographed gave a 100.3 percent recovery in the feces of the total hay pigments involved. When the composite hay pigments of June, 1952 are compared with a composite feces pigment curve (representative sample from 5 days' collection from cow 647), a 94.3 percent recovery of the feces pigments resulted. These three examples average 97.4 percent recovery. This evidence would tend to indicate a low digestibility of the pigments chromatographed and studied.

In order to show which ethyl alcoholic extract fraction was responsible for the pigment concentration at 415 mμ, figure 2 is presented. It will be noted that the largest percentage of the pigment concentration was extracted from the 80 percent ethyl alcohol fraction.

Discussion

Plant pigments exist in chemical equilibrium. When such pigments are exposed to the activity of the intestinal tract, a shift in equilibrium and also a degradation of these pigments occur. Although absorption occurs to some extent, as well as the re-excretion of certain animal and plant porphyrins into the intestine, the composite picture of plant pigments may retain its *status quo*. This idea would help to substantiate the basis for the chromogen marker technique, while on the other hand, it invalidates the idea of an isobestic point existing for most hays and the corresponding feces at 406 mμ. The main portion of the pigments involved at 415 mμ was extracted from the 80 percent EtOH fraction in both the hay and feces. Although some overlapping of individual absorption curves will occur at 415 mμ between the 80 and 70 percent fractions, the predominating concentration of "pheophytin" in the 80 percent fraction may offset this difficulty.

In order to determine the absorption of chlorophyll or its products from the intestinal tract, it is recommended that macrokjeldahl nitrogen be determined on the composite ether extract fractions of the hay

and feces pigments from a digestion experiment. Preliminary work along this line indicates that no foreign nitrogenous material is removed in the process of chromotography, but the data concerning pigment nitrogen absorption were inconclusive. An alternate technique would be a spectrophotometric study of the porphyrins present.

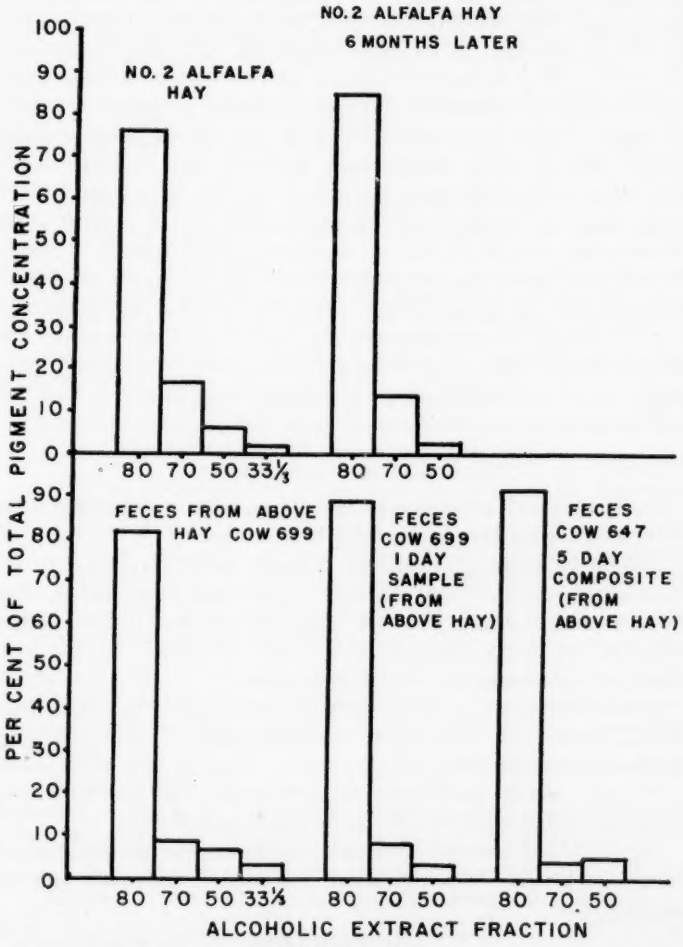

Figure 2. Percent of total pigment concentration of each alcohol fraction at 415 millimicrons. Pigments extracted with ether from 80, 70, 50, 33 1/3 percent ethyl alcohol extract.

Plant pigments and their value as a marker may be enhanced by their equilibrium characteristics. If the chlorophyll pigments can be forced in one direction by treating them with acid or alkali, a consolidation of these pigments might occur, thereby yielding a more stable condition from which a better marker might evolve. Oxalic acid might produce the required results in this case (Joslyn and MacKinney, 1938; Wilstätter and Stoll, 1928). Also the $CaHPO_4 \cdot 2H_2O$ used as an adsorbent in the chromatographic separation of the pigment has a pH of 6.5 and is probably instrumental in the conversion of chlorophyll to "pheophytin." Further evidence is needed to clarify the pigment chromogen marker technique, for an extremely complex picture exists between the pigments found in both hay and feces. Auto-oxidation, photo-oxidation, reduction, enzymolysis and many other factors play an important part in development of the pigment equilibrium relationship as it exists in the plant and animal body. Regardless of these facts, the similarity of the two composite absorption curves as shown in figure 1, indicates that this technique may eventually be employed in the establishment of a digestion trial marker. The development of these types of absorption curves in feed and feces would have its advantages over the isobestic point technique as developed by Reid *et al.* (1950).

Summary and Conclusions

Carotenoids and xanthophylls are too unstable and too digestible to be used for markers in digestion trial studies.

Chlorophyll, although definitely changed, may be retained in the digestive tract in sufficient quantity to be used as a marker. Preliminary work indicates that the composite pigment picture in hay and feces contains a similarity which lends authenticity to the basic theory of the chromogen marker technique.

Pigment nitrogen or the porphyrin content of hay and feces should be studied to substantiate the indigestibility of chlorophyll and its degradation products.

No one pigment can be used as a digestion trial marker because of the dynamic conditions existing in the overall picture.

The individual pigments, although varying greatly between hay and feces, may produce composite absorption curves comparable with one another.

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REPEATABILITY OF PRODUCTION IN RANGE BEEF COWS [1]

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IN order to estimate the effectiveness of selection for production, it is necessary to know the repeatability of the traits which measure production. Also, repeatability of the traits must be known in order to compare accurately the producing ability of cows with varying numbers of records. In experimental work where group comparisons are made, repeatability estimates are very useful in order to determine the best method of allotment.

Knapp *et al.* (1942) estimated that about 19 percent of the variance in birth weight and about 20 percent of the variance in weaning weight were due to differences between cows. Koger and Knox (1947) found an average correlation of 0.49 between weaning weights of all adjacent calves by the same cow, and of 0.24 between grades of first and second calves. Gregory *et al.* (1950) determined the correlation and regression between first and second, first and third, and second and third records for weaning weight, birth weight, and gain from birth to weaning. Estimates of repeatability ranged from 0.35 to 0.50 for weaning weight, from 0.38 to 0.57 for gain from birth to weaning, and from —.12 to 0.24 for birth weight. Koch (1951) determined the intraclass correlation between weaning weights of calves by the same cow. His estimate of the portion of the variance in weaning weights which was due to permanent differences between cows was 0.52.

Materials and Methods

The data used in this study were birth weights and weaning weights of calves produced in the experimental herd of grade Hereford cows at Stillwater and in the experimental herd at the Fort Reno Experiment Station. There were birth weights on 620 calves and weaning weights on 603 calves produced by 151 cows at Stillwater from 1944 through 1951, and birth weights and weaning weights on 98 calves produced by 49 cows at Fort Reno during 1950 and 1951. The cows in the herd at Stillwater calved first at 3 years of age, while the group at Fort Reno calved first as two-year-olds.

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Most of the calves were dropped during February, March, and April, although a few were dropped in January and May. The calves were weighed within 24 hours of birth. Calves were dehorned and the males castrated in late April with the exception of the late calves, which were not dehorned nor castrated until fall. With the exception of one group of 26 calves which were creep-fed during the summer of 1951, all calves were with their dams from birth to weaning without access to any feed other than their mothers' milk and what grass they would eat. Each year, all calves were weaned at the same time, usually in October.

Birth weights were corrected for the effects of sex of calf, age of dam, and year. Weaning weights were corrected for age of calf, sex of calf, age of dam, and year. Repeatability of birth weights, weaning weights, and gains from birth to weaning were determined by two methods: intraclass correlation between calves by the same cow, and the regression of subsequent records on earlier records by the same cow.

Correction of Data

Age of Calf

The age at weaning of calves included in the study varied from 120 to 260 days. Weaning weights were divided into subclasses according to sex of calf, age of dam, and year; the regression coefficient was then calculated on an intraclass basis as shown in table 1.

Weaning weights were adjusted to a standard age of 210 days, using methods described by Whatley and Quaife (1937) and Phillips and Brier (1940), as shown in the following formulas:

$$\text{Age Intercept} = \text{Average Age} - \frac{(\text{Average Weaning Weight})}{(\text{Regression Coefficient})}$$

$$\text{Age Intercept} = 210 - \frac{(432)}{(1.46)} = -86$$

$$\text{Corrected Weight} = \text{Actual Weight} \times \frac{(\text{Standard Age} - \text{Age Intercept})}{(\text{Actual Age} - \text{Age Intercept})}$$

$$\text{Corrected Weight} = \text{Actual Weight} \times \frac{(296)}{(\text{Age} + 86)}$$

In using this method of correction, it is assumed that growth is linear during that portion of the growth curve to which corrections are applied. Growth curves were plotted for five groups of calves produced in 1948, 1950, and 1951. Growth was very nearly linear during the portion of the curve to which corrections were applied. However, these curves were obtained from groups of calves during

the best years as far as weaning weight was concerned, and may not be typical of the growth under less favorable late summer grazing conditions.

Sex of Calf

The average difference between males and females at birth was 4.4 pounds, and at weaning 24.6 pounds. The weights were corrected to a male equivalent by adding 4 pounds to the birth weights of all heifers and 25 pounds to their age-corrected weaning weights.

TABLE 1. REGRESSION OF WEANING WEIGHT (Y) ON WEANING AGE (X)

Source of Variation	D.F.	Sx^2	Sxy	b
Total	609	432,291	577,671	1.34
Subclasses	110	138,235	148,943	....
Intraclass	499	294,056	428,728	1.46

Age of Dam

After weights had been corrected for age of calf and sex of calf, the effect of age of dam was determined, and weights were corrected to a mature cow equivalent. All cows 5 years and older were considered as mature. Weaning weights were adjusted to a mature cow equivalent by adding 35 and 15 pounds to the weights of calves from three- and four-year-old cows respectively. Birth weights were adjusted by adding 4 and 2 pounds to the weights of calves from three- and four-year-old cows respectively. Correction in this manner removed 82 percent of the variance in weaning weights and 62 percent of the variance in birth weights due to differences in age of dam.

Year

The effect of year on weaning weights seemed largely to be due to grazing conditions, which in turn seemed to be due largely to the total rainfall during July and August, but the effect on birth weights was rather inconsistent. The effect of year on birth weights and weaning weights was removed by correcting the weights of calves in each year to the average of all years.

Estimates of Repeatability

Intraclass correlation and the regression of subsequent records on the first record were used to determine the repeatability of weaning

weight, birth weight, and gain from birth to weaning in the Stillwater data. For the data from Fort Reno, simple correlation coefficients were calculated between first and second records for all three traits.

Weaning Weight

Weaning weights (corrected for age of calf, sex, age of dam, and year) were analyzed to determine the portion of variance due to permanent differences between cows. The analysis of variance is shown in table 2.

TABLE 2. ANALYSIS OF VARIANCE OF CORRECTED WEANING WEIGHTS

Source of Variation	D.F.	Sum of Squares	Mean Square	Mean Square Is an Estimate of
Total	602	1,093,312	....	.....
Cows	150	623,657	4,158	$\sigma^2e+4^a\sigma^2c$
Calves by the same cow	452	469,655	1,039	σ^2e

[a] The average number of calves per cow was 4.

The value of σ^2_e was 1,039 and this represents the variance between calves by the same cow. The increase in variation between calves having different dams, σ^2_c, was calculated to be 780, using methods described by Winsor and Clarke (1940) for computing variance components in a one-way classification.

Repeatability of weaning weight can be calculated as the ratio of variance between cows to the total variance as follows:

$$\text{Repeatability} = \frac{\sigma^2_c}{\sigma^2_c+\sigma^2_e} = \frac{780}{1819} = 0.43$$

The upper and lower limits at the 5 percent confidence interval were calculated as outlined by Fisher (1936) and were 0.55 and 0.29 respectively.

To determine the regression of later weaning weights on the first weaning weight, cows were divided equally into a high group and low group on the basis of weaning weights of their first calves. The average weight of calves from the two groups of cows, and differences between the two groups are shown in table 3.

Regression of second records toward the herd average can be determined by dividing the difference between weaning weights of second

calves from the two groups of cows by the difference between weaning weights of their first calves.

$$\text{Regression} = \frac{32.4}{63.2} = 0.51$$

Using the average weaning weight of all calves later than the first, the regression is as follows:

$$\text{Regression} = \frac{31.1}{63.2} = 0.49$$

TABLE 3. AVERAGE WEANING WEIGHTS (IN POUNDS) OF CALVES FROM HIGH AND LOW GROUPS OF COWS SELECTED ON THE WEIGHT OF THEIR FIRST CALVES

Item	Total No. of Calves	High Cows	Low Cows	Difference
Average weaning wt. of first calves	150	489.6	426.4	63.2
Average weaning wt. of second calves	150	471.2	438.8	32.4
Average weaning wt. of all later than first	446	469.5	438.4	31.1

Birth Weight

Repeatability of birth weight was determined by the same two methods. The intraclass correlation between weights of calves by the same cow was 0.18 while the regression of all subsequent records on the first was 0.14.

Repeatability of gain from birth to weaning was 0.38 by both methods.

The correlations between first and second records for the cows at Fort Reno were as follows: for weaning weight 0.66, for birth weight 0.25, and for gain from birth to weaning 0.69. These cows all calved first as two-year-olds and produced calves in consecutive years.

Discussion

The corrections as used were made as simple as possible in order that they might be usable by cattlemen. The regression of weaning weight on age at weaning of 1.46 is not directly comparable to those of other workers, due to differences in average age and average weight at weaning. Therefore, the use of this regression coefficient is only applicable under similar conditions of environment and where cattle are handled similarly to those in the present study. The difference in weaning weight in favor of males over females is in agreement with

differences found by Koger and Knox (1945a, b), Knapp *et al.* (1942), and Koch (1951). However, Gregory *et al.* (1950) failed to find a significant difference between sexes for weaning weight, and Sawyer *et al.* (1949) found heifers to be heavier than males. Dawson *et al.* (1947), Gregory *et al.* (1950), and Burris and Blunn (1952) all found about the same difference between sexes for birth weight as was found in the present study, about 4 to 5 pounds in favor of males.

The effect of age of dam on weaning weight follows about the same trend as found by Knapp *et al.* (1942), Knox and Koger (1945), and Koch (1951). However, the old cows (10-, 11-, 12- and 13-year-olds) in the present study produced heavier calves than would be expected according to the trend of the production curve. The apparent increase in weaning weight of calves from older cows was attributed largely to chance in sampling. Correcting the weights of calves from three- and four-year-old dams was fairly effective in removing the variance in weaning weight due to differences in age of dam, but was much less effective in removing variance in birth weight due to differences in age of dam.

The estimates of repeatability obtained in the present study are in fairly close agreement with those reported from other studies. For weaning weight, estimates of 0.43 and 0.49 were obtained, as compared to estimates of 0.49 by Koger and Knox (1947), 0.52 by Koch (1951), and estimates ranging from 0.37 to 0.50 by Gregory *et al.* (1950). The only other estimates of repeatability of gain from birth to weaning are those of Gregory *et al.* (1950) ranging from 0.38 to 0.57, as compared to estimates of 0.38 in the present study. Estimates of repeatability of birth weight are 0.18 and 0.14, as compared to those of Gregory *et al.* (1950) ranging from —.12 to 0.24.

The repeatability fraction includes all differences due to permanent differences between cows, and thus measures the accuracy of past production in predicting future production of the same herd of cows. Since some of the permanent differences between cows may be non-transmissible, repeatability will be at least as great, and probably greater, than heritability. Thus, repeatability can be used to indicate the immediate gain in future production of a herd which may be achieved by selection; but to the extent that it contains non-hereditary differences between cows, it tends to overestimate what can be gained in future generations by selection.

Data were not available in the present study to obtain an estimate of heritability, so a direct comparison of repeatability and herita-

bility could not be made. However, Lush and Arnold (1937) obtained both estimates from the same data for butterfat production in dairy cattle. Their estimates were 0.43 for repeatability and 0.28 for heritability. Thus, in their study, heritability was about $\frac{2}{3}$ of repeatability.

If any variation is due to temporary influences, producing ability of cows can be more accurately estimated from an average of several records than from a single record. Deviations due to temporary conditions are likely to be random in direction. Thus, when averages are used, the deviations due to temporary conditions tend to cancel each other somewhat. According to Lush (1949), the most probable producing ability of the cow equals the herd average $+ \frac{nr}{1+(n-1)r}$ times (her own average minus the herd average). Here n is the number of records and r is the repeatability of the trait under consideration. The fraction $\frac{nr}{1+(n-1)r}$ shows how much confidence can be placed in the cow's record as an indication of her producing ability.

With repeatability of weaning weight as high as reported here, the very low producers can be culled after their first calves' weights are available, with little danger of culling good cows. Cows at either extreme, either high or low producers, contribute much more to the repeatability of weaning weight than those fairly near the average. Therefore, if only a few cows are to be culled, the first record should be fairly accurate.

If birth weight is used as a measure of a cow's production, selection on the basis of the first record would not be very accurate. However, birth weight is not nearly as useful as weaning weight as a measure of production. Gain from birth to weaning makes up the greatest portion of weaning weight, so is quite comparable to weaning weight as a measure of production. Likewise, the repeatability of gain from birth to weaning seems to be quite comparable to that of weaning weight.

In experimental work in which weaning weight of calves is used as a measure of cow performance, the allotment of cows can be improved if something is known about their producing ability before the experiment is begun. The most accurate method of taking into account previous knowledge is the use of a covariance analysis and the adjustment of lot means for past production. Approximately 16 to 25 percent of the variance in weaning weights can be removed, since the correlation between past and future production seems to be somewhere around 0.4 to 0.5.

An estimate of repeatability is merely a description of a certain population under certain conditions of environment. The application of the estimate, therefore, is limited to those situations where cattle are handled under conditions similar to those from which the estimate was obtained, and weights corrected for the same sources of variation. However, the similarity of different estimates of repeatability of the various traits in beef cattle indicates that the same estimate can be used with confidence under quite a variety of conditions.

Summary and Conclusions

Data investigated were 603 weaning weights and 620 birth weights of calves produced by 151 range Hereford cows in the experimental herd at Stillwater and the weaning weights and birth weights of 98 calves from 49 cows in the experimental herd at the Fort Reno Experiment Station.

Weaning weights were corrected for age of calf, sex of calf, age of dam, and year. Birth weights were corrected for sex of calf, age of dam, and year. Repeatability of weaning weight, birth weight, and gain from birth to weaning were determined by two methods for the Stillwater data: intraclass correlation between calves by the same cow, and regression of subsequent records on earlier records by the same cow. Estimates of repeatability were as follows: for weaning weight 0.43 and 0.49, for birth weight 0.18 and 0.14, and for gain from birth to weaning 0.38. Correlations between first and second records for all three traits were determined for the group of cows at Fort Reno. Correlations were as follows: for weaning weight 0.66, for birth weight 0.25, and for gain from birth to weaning 0.69.

The results indicate that considerable progress can be made in selecting cows on the basis of their first records, particularly by using weaning weights. In experimental work, consideration of differences in inherent producing ability of cows may add considerably to the probable accuracy of the results.

More information is needed to determine what portion of repeatability is due to the additive effects of genes, in order to predict more accurately what gain can be expected in future generations from selecting cows for productivity.

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COMPARATIVE PERFORMANCE OF BEEF CALVES WEANED AT 90 AND 180 DAYS OF AGE[1,2]

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THE genetic components of variation in gain and in weight have been found to increase rapidly during the first 168 days post-weaning in beef calves (Knapp and Clark, 1947; Knapp and Woodward, 1951). These reports imply a post-weaning non-test feeding period of about 168 days followed by a test period of 100 to 150 days. Thus, an animal would be about 16 months old at the completion of the test, assuming it was weaned when 6 months old.

As pre-weaning environmental influences are quite evident for a reasonably long time post-weaning, it may be that by weaning calves earlier than 6 months of age a satisfactory test might be secured while the calf is still relatively young.

The present study is based on the assumption that a calf weaned at a given age requires a preliminary feeding period of a certain length of time (T) prior to test feeding to eliminate preweaning influences. A test feeding period of duration (X) is then needed to evaluate comparative performance. The value (T) is theoretically the quotient of the accumulation of preweaning influences (A) divided by (R) the rate at which these influences are dissipated.

Various situations might be possible when comparing calves weaned at 90 and 180 days of age. T_{90} may be less than, equal to, or greater than T_{180}, and for each of the possible T relationships, X_{90} may be less than, equal to, or more than X_{180}. A situation where $T_{90}<T_{180}$ and $X_{90}<X_{180}$ would result in the quickest test possible. If early weaning is found to give as reliable a test for rate and/or economy of gain at an earlier age than 6–7 month weaning, the process of selecting beef breeding stock for these traits could be speeded up where time is frequently of practical importance.

The present project is in a sense divided into two phases: (a) management and feeding problems, and (b) genetic studies. Phase (a)

[1] Scientific Article No. A394, Contribution No. 2413 of the Maryland Agricultural Experiment Station (Department of Animal Husbandry), University of Maryland.

[2] This work was supported in part by funds provided through the Southern Regional Beef Cattle Breeding Project, S-10.

[3] Prepared in part from University of Maryland M.S. thesis of Mr. John Buric. The writers are indebted to Dr. J. E. Foster, Head, Department of Animal Husbandry for making this work possible.

has been completed and is the basis of this report. Data for phase (b) have been accumulated during phase (a) and continue to be collected.

Materials and Methods

Sixty-two purebred Aberdeen Angus and Hereford calves (steers and heifers) were weaned at 90 or 180 days of age as shown in table 1.

The 1949 calves were arranged by birth date, within breed, and assigned to their respective weaning groups at random. In 1950,

TABLE 1. NUMBERS OF CALVES BY WEANING AGE, YEAR OF BIRTH, BREED AND SEX FED INDIVIDUALLY FROM WEANING TO 370 DAYS OF AGE

Group Number	Year Started	Weaning Age (Days)	Ages While on Feed							
			90–180 Days				180–370 Days			
			Angus		Hereford		Angus		Hereford	
			M	F	M	F	M	F	M	F
1 [1]	1949	90	5	2	1	4	5	2	1	4
2 [2]	1949	180	..	..	..	..	..	2	..	2
3 [1]	1950	90	4	7	3	1	4	7	3	1
4 [2]	1950	180	..	..	..	..	4	1	1	2
5 [1]	1951	90	7	1	..	3	7	1	..	3
6 [2]	1951	180	..	..	..	..	7	2	1	2

[1] Groups 1, 3, and 5 were composed of calves fed continuously from 90 to 370 days of age.
[2] Groups 2, 4, and 6 were composed of calves fed continuously from 180 to 370 days of age.

sibs or maternal half-sibs of 1949 calves were placed in the weaning age group opposite to their 1949 sib or maternal half-sibs, and the non-maternal half-sibs were assigned at random. The 1951 calves were placed in weaning age groups opposite to a 1949 or 1950 sib or maternal half-sib or at random if it were not a maternal half-sib. Full- or half-sibs were not assigned to a particular weaning age group on the basis of the performance of a predecessor. All Herefords except one and all 1950 and 1951 Aberdeen Angus calves were paternal half-sibs within breed.

Spring calves were used and they were allowed to stay with their dams, on pasture, until weaning. Creep feeding was not practiced. Male calves were castrated when 2 to 3 weeks of age. All calves were weighed at birth, 90, and 180 days of age. In addition, weights were secured at the end of each week for 4 weeks post-weaning and at the end of each 28-day feeding period until the calves were 370 days of age. Calves weaned at 180 days of age were weighed also on

their 202nd day, the latter age being the initiation of their first 28-day feeding period. All weights were taken at approximately 2 p.m. without fasting prior to weighing.

The calves were fed individually post-weaning and feed records were kept on a 24-hour basis. Chopped hay and grain were fed separately and *ad libitum* at all times. All individuals were placed on feed in the afternoon (4 p.m.) of the day of weaning. Calves weaned at 90 days of age were fed grain and hay at 4 p.m., 6 a.m., and at noon until they were 180 days of age. From 180 to 370 days of age all calves were fed grain and hay at 4 p.m. and 6 a.m. with the exception of the 1951 calves which were fed hay only at noon in addition to the other two feedings. The steers and heifers were allowed to feed in individual tie-stalls for at least 1 hour at each meal. They were then returned to common loafing pens and paddocks where water, salt and bone meal were available.

A commercial calf starter of known composition was fed from 90 to 118 days. Beginning with the fifth week, the calf starter was gradually superseded by the grain mixture given below, so that at the beginning of the eighth week calf starter was no longer being fed. The following grain mixture was given all calves between the ages of 139 and 370 days:

U. S. No. 2 yellow corn, coarsely cracked	1000	pounds
U. S. No. 2 white oats, crimped	521	"
Wheat bran	174	"
Cottonseed oil meal	174	"
Alfalfa leaf meal	87	"
Linseed oil meal	44	"
Salt, iodized	10	"
Steamed bone meal	10	"

U. S. No. 1 alfalfa hay was used from 90 to 180 days and U. S. No. 2 alfalfa hay from 180 to 370 days. The hay, calf starter, and grain mixture were analyzed for moisture, protein, fat, fibre, and ash by the State of Maryland Inspection and Regulatory Service, using methods recommended by the Association of Official Agricultural Chemists.

Calculation of feed consumption was made on the basis of total digestible nutrients (TDN) consumed. The percent TDN for the calf starter and grain mixture was computed by use of the actual amounts of the various ingredients and the TDN values given for each feed by Morrison (1948). The TDN values for the hays were secured by comparing the chemical analysis of the hay used with those given by Morrison (1948). The percent TDN values used for

all calculations were: hay, 51.5; calf starter, 73.3; and grain mixture, 74.5.

Data were analyzed by methods given by Snedecor (1948). Terms statistically significant will apply to situations where p is 0.05 or less and highly significant where p is 0.01 or less. The main approaches used were (a) analysis of variance primarily on an age or 28-day feeding period basis, (b) covariance to test the influence of a probable factor on the main one in question, and (c) correlation analysis either on an age or 28-day feeding period basis or on a grouping of feeding periods. In the latter case all data were used by calculating on an individual calf-per-period basis rather than by use of period means.

Results and Discussion

A summary of average weights at various ages by breeds, sexes and weaning age groups is shown in table 2. Differences between group means were tested by use of analysis of variance, the sources of variation being designated as given in table 3. Analyses were made at each age at which weights are indicated in table 2.

Statistically significant differences in weight were not found among years or between breeds in the data for the heifers or steers, or in the combined heifer-plus-steer data. No significant differences in weight between weaning age groups were found in the steers. The only significant differences between weaning age groups occurred in the females, and heifer-plus-steer data, at 146, 174, and 180 days of age. The group which differed from all of the rest was the 90-day female Angus. It may be noted from table 2 that this group weighed the least at birth and maintained its lighter weight all during the trial. No identifiable reason was found for this phenomenon. The animals were allocated as previously stated, and there were no significant differences among years within the 90-day Angus female group so the difference was not due to light-weight calves in any particular year. The result might be due to a breed-sex condition as, in general, the females tended to be lighter in weight. The situation could be, and may well be, due to sampling. A covariance analysis using 118- and 146-day weights indicated that the difference found at 146 days was not attributable to any great extent to the variations in the 118-day weights; however, a covarance analysis involving 146- and 180-day weights indicated that practically all of the variance in the 180-day weights was attributable to variations in the 146-day weights. Figure 1 helps to explain the transient difference in weight since the 90-day weaned group gained much less from 90–146 days than did the other

TABLE 2. SUMMARY OF AVERAGE WEIGHTS AT VARIOUS AGES, AND AVERAGE TDN CONSUMPTION FOR FEEDING PERIODS BY WEANING AGE GROUPS, BREED, AND SEX

Weaning Age	Breed	Sex [1]	No.	Average Weights at Days of Age as Indicated												
				Birth	90	118	146	174	180	202	230	258	286	314	342	370
(days)																
90	Angus	F	10	57	185	210	252	293	303	333	375	417	460	500	549	583
180	Angus	F	5	62	202	249	309	357	363	595	447	473	523	574	612	656
90	Hereford	F	8	62	202	239	282	328	340	381	425	470	518	564	611	654
180	Hereford	F	6	63	183	228	285	332	342	364	417	448	493	535	582	626
90	All	F	18	59	193	223	266	309	319	354	398	441	486	529	577	615
180	All	F	11	62	192	238	296	343	352	378	431	459	507	553	596	640
90	Angus	S	16	64	211	245	287	334	345	384	435	485	533	587	628	676
180	Angus	S	11	63	202	250	303	351	359	382	430	476	526	571	628	670
90	Hereford	S	4	68	200	228	278	327	338	375	424	482	528	587	648	702
180	Hereford	S	2	72	216	277	339	393	402	410	463	509	579	629	677	733
90	All	S	20	65	209	242	285	332	343	383	433	485	532	587	632	681
180	All	S	13	64	204	254	309	357	365	386	435	481	534	580	635	679
90	All	F+S	38	62	201	233	276	321	332	369	416	464	510	559	606	650
180	All	F+S	24	63	198	247	303	351	359	382	433	471	522	567	617	661
90+180	All	F+S	62	63	200	238	286	333	342	374	423	467	515	562	610	654

Weaning Age	Breed	Sex [1]	No.	Average TDN Consumption for Inclusive Ages as Indicated									
				90–118	118–146	146–174	174–202	202–230	230–258	258–286	286–314	314–342	342–370
90	Angus	F	10	66	134	166	186	202	204	214	229	250	271
180	Angus	F	5	..	...	...	...	222	200	236	260	279	298
90	Hereford	F	8	88	141	167	195	211	205	225	242	262	281
180	Hereford	F	6	..	...	...	...	199	188	220	228	252	263
90	All	F	18	76	137	166	190	206	205	219	235	255	275
180	All	F	11	..	...	...	...	209	194	227	243	264	279
90	Angus	S	16	85	154	177	215	234	240	248	263	280	301
180	Angus	S	11	..	...	...	...	199	233	252	262	284	302
90	Hereford	S	4	73	147	176	196	217	227	237	276	275	311
180	Hereford	S	2	..	...	...	...	175	232	271	266	279	329
90	All	S	20	82	153	177	211	230	237	246	266	279	303
180	All	S	13	..	...	...	...	195	232	255	262	283	306
90	All	F+S	38	79	145	172	201	219	222	233	251	268	290
180	All	F+S	24	..	...	...	...	202	215	242	254	275	294
90+180	All	F+S	62	79	145	172	201	212	219	236	252	271	291

[1] *F* represents heifers; *S* represents steers.

group, probably due to the effects of weaning. Weaning caused a transitory reduction in immediately subsequent gains, and both groups gained essentially at the same rate during the 28-day period including or immediately following weaning.

Statistically significant differences were found in weight between sexes at two main periods: (a) birth to 118 days, and (b) 258 to 314 days. There were in addition a significant difference at 180 days and a highly significant difference at 370 days. The group which caused these differences was the 90-day Angus female. Covariance analyses were made on all data per period (not just the Angus female

TABLE 3. SOURCES OF VARIATION AND DEGREES OF FREEDOM AS USED FOR ANALYSIS OF VARIANCE

Source of Variation	Degrees of Freedom		
	Females	Males	Females+Males
Ages	1	1	1
Years	2	2	2
Breeds	1	1	1
Sexes	..	..	1
Error	24	28	56
Total	28	32	61
N=	29	33	62

group) involving the weights for a particular age and the weights at the adjacent previous age. Indications were that previous-age weights contributed highly in causing the significance in differences at 90, 118, 180, 286, and 314 days, while other factors were seemingly more important causes of the significance of difference at 146, 258, 342, and 370 days of age.

Growth as measured by gross weight may be studied as gain per period or by progressive gross weights. The latter measure was used for the data summarized in table 4. The regression of weight on age was essentially the same for the females weaned at 90 days during the two age intervals indicated. The females weaned at 180 days grew more slowly after weaning and the two breeds paralleled their actions in this respect. It is also of interest to note that the relationship of the Angus and Hereford females within weaning age groups maintained their relative differences. More reliance on the results of the 90-day and 180-day Angus-plus-Hereford data (lines 5 and 6) seems justifiable because of a larger number of animals involved and the parallelism between groups.

TABLE 4. ZERO ORDER CORRELATION (r) AND LINEAR REGRESSION (b) COEFFICIENTS OF WEIGHT AND AGE BY WEANING AGE, BREED AND SEX GROUPS

			Inclusive Ages							
			118–180 Days				202–370 Days			
Weaning Age	Breed	Sex [1]	N	r**	b	Std. Error of Estimate for Regression	N	r**	b	Std. Error of Estimate for Regression
(days)										
90	Angus	F	40	0.822	1.48	26.06	70	0.914	1.51	37.88
90	Hereford	F	32	0.947	1.62	13.96	56	0.958	1.64	27.86
180	Angus	F	20	0.946	1.86	16.52	35	0.922	1.55	37.50
180	Hereford	F	24	0.707	1.82	47.01	42	0.721	1.54	84.66
90	Angus + Hereford	F	72	0.819	1.54	27.04	126	0.893	1.57	44.43
180	Angus + Hereford	F	44	0.778	1.84	37.51	77	0.788	1.54	68.43
90+180	Angus + Hereford	F	116	0.770	1.66	34.15	203	0.844	1.56	55.67
90	Angus	S	64	0.840	1.60	26.00	112	0.921	1.74	41.62
90	Hereford	S	16	0.831	1.76	31.16	28	0.967	1.96	29.78
180	Angus	S	44	0.678	1.76	48.15	77	0.872	1.73	55.01
180	Hereford	S	8	0.620[a]	2.03	73.10	14	0.817	1.93	82.57
90	Augus + Hereford	S	80	0.834	1.64	27.02	140	0.930	1.78	39.67
180	Angus + Hereford	S	52	0.652	1.80	52.69	91	0.852	1.76	61.32
90+180	Augus + Hereford	S	132	0.725	1.70	40.20	231	0.897	1.77	49.09

** All correlations statistically significant at the 1% level except *a*.
[1] *F* represents females, *S* represents steers.

A sex difference is indicated in table 4 in that the steers reacted in a manner the reverse of the females (lines 8 through 13). The number (N) shown in table 4, as in some subsequent tables, results from the use of all animals for a particular age or feeding period. For example, in the 90-day female Angus group, $N=40$ is secured by use of the data of 10 animals for four different ages (118, 146, 174, and 180 days). Seven ages were included in the 202–370 day period. Deletion of the 90–118 and 180–202 day periods for the 90- and 180-day weanling groups, respectively, was used in many instances in an attempt to remove the immediate effects of weaning.

The results in table 4, as elsewhere, are based on small numbers

TABLE 5. ZERO ORDER CORRELATION (r) AND LINEAR REGRESSION (b) COEFFICIENTS FOR WEIGHT AND AGE FOR FEMALES BY WEANING AGE AND BREED

		Inclusive Ages 202–314 Days			
Weaning Age	Breed	N	r**	b	Std. Error of Estimate for Regression
(days)					
90	Angus	50	0.866	1.49	34.81
90	Hereford	40	0.944	1.64	23.39
180	Angus	25	0.876	1.55	35.24
180	Hereford	30	0.626	1.50	76.47
90	Angus + Hereford	90	0.838	1.56	40.67
180	Angus + Hereford	55	0.701	1.52	62.27
90+180	Angus + Hereford	145	0.770	1.54	50.95

** All correlations statistically significant at the 1% level.

of animals especially when sub-groupings are used; therefore, the results are interpreted to be indicative rather than final. It is of interest to note in table 4, however, that the standard errors of estimate are usually lower (frequently about ⅓ to ½) for the animals weaned at 90 days than for the 180-day weaned group (note especially lines 5, 6, 12 and 13). Data for females only through the period of most uniform gain (see figure 1) are given in table 5. The results are, of course, in essential agreement with those in table 4.

Rate of gain was analyzed not only in the manner shown in table 4 but also by analysis of variance (table 3). A summary table for average gains per period is not given as it can be calculated from table 2 and the essential results may be seen quickly in figure 1. Covariance studies were made on gain wherein the weights at the

beginning of a 28-day period and gains during the same period were involved. Among the females, highly significant differences in gain (analysis of variance) between weaning age groups were found in the 90–118 and 118–146 day periods and significant differences existed at the ages of 174–202 and 230–258 days. Covariance analysis indicated that at the 90–118, 118–146 and 230–258 day periods, weight at the start of a period had a very slight effect on the gain during the period; for the 174–202 day interval, covariance reduced the level of significance of the variance ratios from slightly over 5 percent to just below that level. Significant differences in gains among years (female analysis) were found at 174–202 and 230–258 days and highly significant differences at the 314–342 day period. Covariance analysis resulted in no significant reductions in "F" value at any of these three periods.

In the steers, highly significant differences were found between mean gains for the weaning age groups at the 90–118 and 174–202 day periods and significant differences at the 118–146 day interval. Again, by covariance analysis, rate of gain during the above-mentioned periods was not found to be associated with the weight at the start of the period. The same held true for the (significant) differences between years for the steers at 90–118 and 286–314 days.

The scattered differences among years in gain at various periods showed no consistency, indicating that the results were not due to any one particular year. The differences among the females appeared to be due to the larger gains of the 1949 calves at 174–202 days, larger gains of the 1950 calves at 230–258 days and to lower gains by the 1951 calves at 314–342 days. For the steers, the low gains of the 1949 group at 90–118 days and the high gains of the 1949 calves at the 286–314 day period seemed to account for the differences.

In the combined steer-plus-heifer data, significant differences among the means of years were found only at 174–202 and 314–342 days, and between sexes at the 230–258 (highly significant), 286–314, and 342–370 day feeding periods. Significant differences in gains existed between weaning age groups at 90–118, 118–146, 174–202 and 230–258 days. With the exception of the difference between sexes at 286–314 days, covariance between the weight at the start of the period and gain during the period caused practically no change in variance ratio for gain. The results were that, except in two of the 21 instances where differences of statistical importance existed in the gain analysis, covariance analysis indicated that the weight of the animal

at the beginning of a period had little or no effect on the gain made during the period.

The lack of association between weight at the beginning and gain during the period was also shown when the weights at the beginning of the period were correlated with gains during the period for all 28-day intervals from 202–342 days inclusive. When the data of the females and the steers were combined, the correlation was 0.004 (n=228) for those weaned at 90 days and 0.01 (n=144) for the

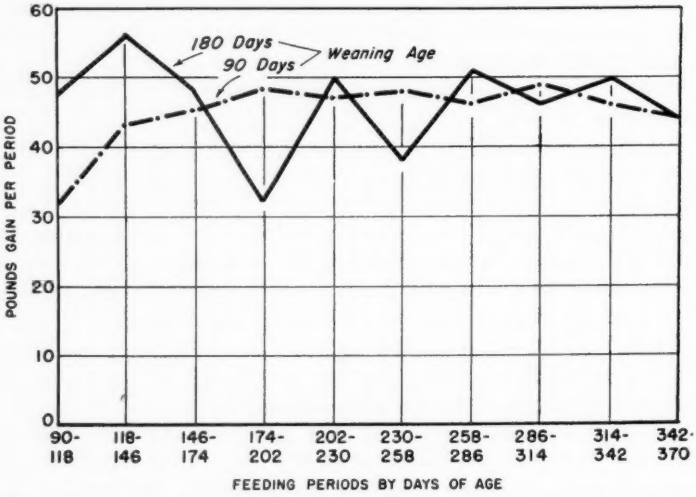

Figure 1. Average pounds gained per 28-day period by calves weaned at 90 and 180 days of age.

180-day group. The correlations of age at the start and gain during the periods were —0.007 and —0.001 for the 90- and 180-day weaning groups (steers plus heifers) respectively. The respective correlations of age and weight were 0.87 and 0.78 for the same age ranges. Figure 1 illustrates the gains per period for calves weaned at 90 and 180 days of age. Different patterns are evident for the two weaning age groups and help to illustrate the low association between age (and indirectly, weight) and gains. Additional evidence for the low degree of association between weight at the start of a period and subsequent gains is presented in table 6, where com-

parisons are made on a period by period basis. The correlations are low numerically and show no uniformity in pattern.

Analysis of variance of TDN consumption by feeding periods indicated only one consistent series of differences; these were differences between sexes from an age of 230 to 370 days (significant at 314–342 days and highly significant at periods beginning at 230, 258, 286 and 342 days). The only feeding period to show many differences

TABLE 6. ZERO ORDER CORRELATIONS (r) BETWEEN GAIN (w) AND WEIGHT AT START (z) OF AGE PERIODS FOR COMBINED STEER AND HEIFER DATA

Age Period	Weaning Age	
	90 Days	180 Days
	(n=38)	(n=24)
90–118	−0.128	0.319
118–146	0.018	0.537**
146–174	0.134	0.310
174–202	0.067	−0.111
202–230	0.203	0.074
230–258	−0.031	0.242
258–286	0.007	0.389
286–314	0.384*	0.137
314–342	0.039	0.460*
342–370	0.305	−0.315

* Statistically significant at the 5% level.
** Statistically significant at the 1% level.

was the 230–258 day period in which significant differences were found among years in both the females and the steers and between breeds for the combined heifer-plus-steer data, and a highly significant difference among years for the combined data. The TDN consumption in the above analysis represented total intake and was used for both maintenance and growth.

In an evaluation of the possible use of weaning calves at 90 days instead of 180 days, no significant differences of any special consequence were found between years, indicating a reasonable degree of reliance might be placed on accumulated data or on making certain year to year comparisons. Weaning age groups differed somewhat in weight at various ages and in rate of gain. The increased uniformity of average gain from one period to another after an age of 146 days, or even after 118 days, suggests that the rate of gain might be

evaluated rather early and quickly in the earlier weaned calves. The increase in average TDN consumption per period is more nearly linear for the 90-day weaned calves than for the others, indicating perhaps an earlier evaluation of economy of gain.

In regard to health and effects on conformation, no differences were noted between the calves weaned at the different ages; reference to a list of calves was necessary in order to ascertain to which weaning group a particular calf belonged. Early weaning is not to be suggested as a practical, routine method for raising beef calves, but it seems to show some promise as a method to evaluate calves at an earlier age. The second phase of this project will have to be completed before a full analysis of the possibilities of this proposed method for evaluating calves can be fully assayed.

Summary and Conclusions

Beef calves were weaned at 90 or 180 days and fed individually until 370 days of age in order to establish feeding and management practices for the earlier weaned calves. Comparisons were also made of the performance of the two groups in view of the possibility of designing a quicker test for rate and economy of gains for beef calves. Heifers weaned at 90 days of age were lighter in weight at 180 days than those weaned later but were of similar weight at 1 year of age. Breed differences were not found in weight and few differences between years were noted. The main difference between the 90- and 180-day weaned groups was in rate of gain, wherein the average gain per 28-day feeding period was much more uniform for the 90-day group. Present indications are that 90-day weaning does not adversely affect beef calves and perhaps an earlier evaluation of rate of gain is possible by early weaning.

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RESPIRATORY ACTIVITY AS AN INDEX OF HEAT TOLERANCE IN JERSEY AND SINDHI × JERSEY (F_1) CROSSBRED COWS [1]

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As part of the Southern Dairy Cattle Breeding Project, Red Sindhi bulls from India were mated with purebred Jersey from the Beltsville herd to see whether the heat tolerance of the former could be combined with the productive capacity of the latter by selective cross-breeding. Studies have been conducted on these animals not only to determine the comparative distribution of these qualities in the offspring, but also to ascertain what animal characteristics are associated with heat tolerance.

One of the characteristics which might be thought to be closely associated with heat tolerance is that of respiratory activity. Cattle, like many other animals, show increased respiratory activity under hot conditions, and the consequently increased air flow over the respiratory passages results in an increased cooling effect.

The amount of cooling obtained can be obtained from the formula:

$$H_r = V[0.00027(t_b - t_a) + 0.00057(p_r - p_a)]$$

where H_r is rate of heat loss from respiratory tract in kcal./hr.
V is respiratory volume in l./hr.
t_b is body temperature in C.°
t_a is air temperature in C.°
p_r is vapor pressure of air leaving respiratory tract (usually taken as saturation vapor pressure at temperature of body) in mmHg.
p_a is vapor pressure of air

Under hot conditions the difference between body temperature and air temperature is sufficiently small for the first term inside the brackets of the equation to be neglected.

It might be thought that the individual showing the higher respiratory activity would have an advantage over the one with a lower

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activity. Numerous reports (Kibler and Brody, 1949, 1950a, b, 1951; Riek and Lee, 1948), however, indicate that the converse is the case, the animals with the higher rate having the poorer heat balance.

While the rate is the aspect of respiratory activity which is most easily observed, especially in the field, it is the volume of air respired which should determine its cooling effect. Laboratory reports (Kibler and Brody, 1949, 1950a, b, 1951; Riek and Lee, 1948) indicate, however, that rate and volume do not increase in the same proportion, and it is known that if they did an alkalosis would tend to develop from a washing-out of carbon dioxide from the blood.

The studies reported here were designed to answer three questions, using somewhat larger numbers of animals than have been used by other laboratory investigators: (a) what is the fundamental relationship of respiratory activity to heat tolerance within a group of dairy cows?; (b) what is the relationship between rate and volume in the response to heat?; and (c) how do the crossbreds differ from the Jerseys in these respects?

Parallel studies are being conducted at the Iberia Livestock Experiment Station, Jeanerette, Louisiana, so that the possible effect of continued exposure to hot condition between tests can be made. The data in this paper deal only with the studies at Beltsville.

Experimental

General

Twenty purebred Jersey cows and 20 Red Sindhi $\times$ Jersey F_1 cross cows were selected from the Beltsville herd to form groups as similar as possible in age, body weight, reproductive state (months in lactation or months pregnant), and level of production (pounds FCM/day). All had completed at least one lactation.

The test animals were placed in the climatic chamber under temperate conditions (dry bulb temperature, 61°–83° F.) on the afternoon before the test day, and offered water, grain, and hay. On the following morning they were fed grain and water but no hay. Three hours after this feeding the animals were removed to the anteroom, which was at "normal" temperature (57°–84° F.), and initial observations made of rectal temperature, respiratory rate, and respiratory volume. They were then returned to the climatic chamber which had, in the meantime, been heated to a temperature of 105° F., with a vapor pressure of 34 mmHg (wet bulb temperature 92° F.). After 3 hours exposure to these conditions, during which time the animals

received no food or water, observations were again made of rectal temperature, respiratory rate, and respiratory volume.[4]

The rectal temperature was determined with a five-inch veterinary thermometer, and respiratory rates were obtained by counting flank movements over a period of 1 to 5 minutes of uninterrupted breathing, depending upon the rate. Respiratory volume was measured by a method to be described below.

At the time of the test each animal was appraised for its "condition" grade. The numerical values for "condition" used in table 1 are quantitative values used to determine the degree of fleshing of cattle. Assessments were made by simultaneous feeling of the fleshing and visual inspection of the areas over withers, mid-region of the 11th and 12th ribs, over the hip bones, over the ends of the 3rd and 4th lumbar vertebrae and around the tail head. Using a scale of 1 to 9 (1 for extreme emaciation, 5 for good average covering and 9 for extreme fatness), each of the five points is evaluated and an average then made to describe the over-all condition of the animal.

Respiratory Volume

To measure the volume of the respired air in animals by direct methods, it is essential that there be no resistance to expiration, that the mask be perfectly comfortable, and that there be sufficient flexibility in attachments to eliminate hazards arising from sudden movements by the animal. The apparatus described here is similar to that described by Kibler (1948) and Kibler and Brody (1950a), differing only in details of the mask, the expiratory valve and control of suction by the exhaust fan (figure 1).

The mask consists of a 4 in. galvanized iron (16 gauge) collar soldered to a 6½ inch disc which carries a centrally placed hose connection 3 in. in diameter. To the metal collar is attached a further 6 in. collar of sheet rubber. Projecting from each side of the metal collar is the housing for an inspiratory flap valve 3 in. in diameter. A thick piece of sponge rubber is tied around the animal's muzzle well above the nostrils, and the rubber collar slipped over this and on up the nose. Two elastic strips are then bound around the rubber collar to make a final seal and keep the mask in place. The weight of the mask is further supported by a strap running around the hose attachment and fastened behind the ears.

[4] The term "respiratory volume" is used in this paper for the volume of air respired per hour (or per minute). The term "tidal volume" is used for the volume of air breathed in and out in one respiration.

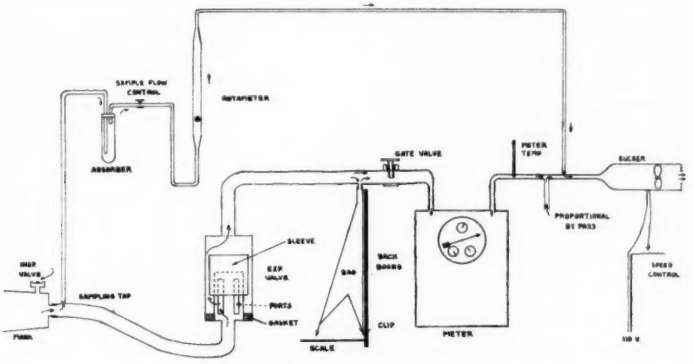
TABLE 1. COMPARATIVE REACTIONS OF JERSEYS AND SINDHI-JERSEY CROSSES TO HOT CONDITIONS [1]

Item		JERSEYS			CROSSES		
Reaction	Time	Milking	Dry	Average	Milking	Dry	Average
Rectal temp. (° F.)	Initial	101.4 (0.146)	101.4 (0.194)	101.4 (0.116)	101.9 (0.130)	101.6 (0.151)	101.8 (0.098)
	Final	104.8 (0.260)	104.1 (0.109)	104.4 (0.245)	103.9 (0.265)	103.4 (0.298)	103.6 (0.199)
	Diff.	3.4 (0.321)	2.7 (0.388)	3.1 (0.236)	2.0 (0.246)	1.7 (0.193)	1.9 (0.152)
Resp. vol. (l./m.)	Initial	96.0 (7.52)	80.8 (10.57)	88.4 (6.42)	80.6 (6.86)	67.3 (8.18)	73.9 (5.29)
	Final	256.2 (11.72)	258.8 (14.12)	255.7 (9.21)	238.0 (6.44)	227.0 (10.67)	232.6 (6.04)
	Diff.	160.2 (8.17)	178.0 (7.19)	169.1 (5.56)	157.4 (6.94)	159.7 (10.46)	158.6 (5.95)
Resp. rate (/min.)	Initial	42.8 (3.78)	42.6 (4.86)	42.7 (2.92)	38.9 (3.69)	34.6 (3.83)	36.8 (2.63)
	Final	140.8 (4.29)	142.4 (5.58)	141.6 (3.29)	153.5 (5.53)	135.7 (4.47)	144.6 (3.97)
	Diff.	98.0 (4.95)	99.8 (4.68)	98.9 (3.23)	114.6 (5.74)	101.1 (5.27)	107.9 (4.02)
Tidal vol. (l./resp.)	Initial	2.31 (0.160)	1.92 (0.143)	2.11 (0.112)	2.18 (0.206)	1.97 (0.151)	2.07 (0.124)
	Final	1.82 (0.072)	1.82 (0.063)	1.82 (0.046)	1.56 (0.049)	1.69 (0.097)	1.63 (0.054)
	Diff.	−0.49 (0.172)	−0.10 (0.081)	−0.29 (0.113)	−0.61 (0.213)	−0.28 (0.156)	−0.45 (0.131)
Milk prod. (lb. FCM)		35.4 (2.47)	....	....	32.6 (3.28)		
Body wt. lb.		1026 (27.6)	956 (40.0)	991 (24.4)	956 (25.1)	946 (46.8)	951 (25.2)
Condition score		5.5 (0.360)	6.1 (0.484)	5.8 (0.474)	6.8 (0.344)	7.7 (0.222)	7.3 (0.223)
Age		4 yr. 7.9 m.	3 yr. 10.2 m.	4 yr. 3.1 m.	4 yr. 2.6 m.	3 yr. 10.4 m.	3 yr. 7.9 m.
Reprod. state (mo. lact./ mo. preg.)		L 4.0 m.	P 4.7 m.		L 2.4 m.	P 4.13 m.	

10 cows in each group. Main figures are means. Figures in parentheses are standard deviations of means.
[1] 3 hours at 105° F. and 34 mmHg vapor pressure.

The hose is attached to an expiratory sleeve valve of low resistance resting on a sponge rubber gasket surrounding the bottom of the chamber. Surrounding the sleeve valve is a galvanized iron housing which conveys the air upwards to a 3 in. pipe running to the meter and a domestic vacuum cleaner. A short bypass to room air is inserted between the meter and the cleaner. The dimensions of this bypass are determined empirically.

From the pipe running between the expiratory sleeve valve and the meter is a branch opening into a 200 liter Douglas bag suspended vertically with one face clamped to a board. By adjustments of the



RESPIRATION CIRCUIT
Figure 1

gate valve, the suction can be regulated so that the bag fluctuates equally on either side of a mark with each expiration and inspiration. When this steady state is obtained, the rate of air flow through the meter just equals the rate of expiration by the animal. To ensure that the free wall of the bag moves as one piece and is not subjected to local bulgings, a piece of stiff but light cardboard is glued to its surface.

Taps are provided at various points of the line for sampling purposes.

When the apparatus is properly constructed and working, trained cattle offer no objection to the procedure and will stand quietly with the mask almost indefinitely. Such training was given all animals used in these studies.

Air volumes reported in this paper have been converted to a standard of 70° F.

Results and Discussion

The superiority of the Sindhi × Jersey crosses over the Jerseys in heat tolerance was in accordance with more extensive observations reported elsewhere (McDowell *et al.*, 1952). That this difference could not be ascribed to variations in other circumstances is indicated (table 1) by the similarity of milk production, age, and reproductive state of the two groups, and the fact that the higher "condition" of

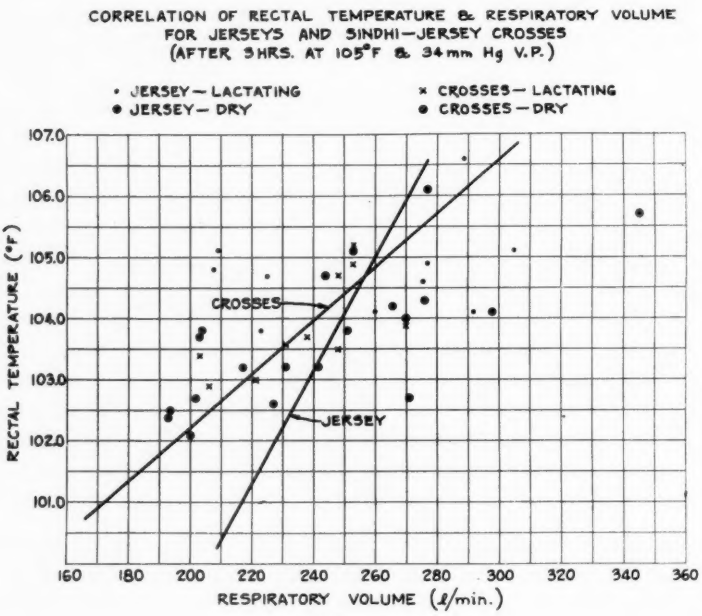


Figure 2

the crosses would have tended, if anything, to make it more difficult for the crosses to maintain a heat balance.

When the test animals were exposed to heat, the respiratory volume of the Jerseys was higher (significant at the 5 percent level) than that of the Sindhi × Jersey crosses and increased to a greater extent. In spite of the additional cooling effect that may be expected from the increased respiratory volume, the rectal temperature of the Jerseys rose above that of the crosses to a highly significant degree. Kibler and Brody (1950b) found an even smaller response by Brahman cows.

The correlation between rectal temperature and respiratory volume (figure 2, table 1) was a positive one, except in lactating animals under moderate conditions (57°–84° F.). The value of the positive correlation coefficient rose to highly significant levels in the crosses, and to significant levels in the dry Jerseys.

From these two sets of observations it is evident that the superior heat tolerance of the crosses could not be ascribed to greater respiratory response. On the contrary, among similar cattle a high respiratory activity is to be regarded as an attempted compensation for a poor heat balance brought about by some other cause.

If the increase in respiratory volume on exposure to heat were effected without changing the depth of respiration, the alveoli would be over-ventilated and some carbon dioxide would be washed out of the blood and body fluids with a consequent alkalosis or actual alkalemia. If, on the other hand, increased respiratory volume is to be obtained solely by ventilating the "dead space," keeping the alveolar ventilation constant, the rate will be increased more rapidly than the volume. To double the volume, keeping alveolar ventilation constant, it may be necessary to quadruple the rate. The relation between required rate, desired volume, and tidal volume for this condition are given by the formulae:

$$R = \frac{V-A}{d} \qquad t = \frac{dV}{V-A}$$

where R is required rate per minute
V is desired respiratory volume in l./min.
A is desired alveolar ventilation in l./min.
d is volume of "dead space" in l.
t is tidal volume in l.

The tidal volume (table 1) was substantially the same in both the Jerseys and the crosses under moderate atmospheric conditions. When the animals were exposed to heat, the tidal volume decreased in both groups but more markedly in the crosses. The crosses thereby secured a further secondary advantage, in that such increase in respiratory activity as did occur was brought about with a still smaller disturbance to normal alveolar ventilation and a much reduced risk of inducing alkalosis. (Solution of the equation given above must wait until a reliable estimate of the "dead space" in cattle is obtained.)

Respiratory rate (table 2) also showed a predominately positive correlation with rectal temperature, but the coefficients were smaller and in no case reached significant levels. The respiratory rate was evi-

dently a less sensitive index of respiratory response to heat than was respiratory volume.

The reactions of lactating animals differed somewhat from those of the dry cows. In both Jerseys and crosses the respiratory volume (table 1) under moderate atmospheric conditions was higher in the lactating than in the dry animals, but this difference largely disappeared when the increased response to heat was induced. Moreover, the correlation between rectal temperature and respiratory volume

TABLE 2. CORRELATION COEFFICIENTS OF BODY TEMPERATURE, RESPIRATORY VOLUME AND RESPIRATORY RATE FOR JERSEYS AND SINDHI JERSEY CROSSBREDS

Type of Animal		Correlation Coefficients		
		Rectal Temp./ Resp. Vol.	Rectal Temp./ Resp. Rate	Resp. Volume/ Resp. Rate
Before exposure to hot room (initial volumes)				
Lactating Jerseys	(10)	—.202	0.187	0.544
Lactating crosses	(10)	—.030	0.170	0.060
Dry Jerseys	(10)	0.256	0.118	0.543
Dry crosses	(10)	0.354	0.379	0.756**
All Jerseys	(20)	0.270	0.298	0.687**
All crosses	(20)	0.262	0.320	0.466
After 3 hours at 105° F. and 34 mmHg V.P.				
Lactating Jerseys	(10)	0.275	—.174	0.055
Lactating crosses	(10)	0.682*	0.581	0.542
Dry Jerseys	(10)	0.606*	0.447	0.759**
Dry crosses	(10)	0.796**	0.044	—.142
All Jerseys	(20)	0.420	0.207	0.640**
All crosses	(20)	0.757**	0.339	0.231

* Significant at the 5% level.
** Significant at the 1% level.

(table 2) was less in the lactating than in the corresponding dry animals, and actually became negative, though not significantly so, under the temperate environmental conditions.

Summary

Twenty purebred Jersey cows and 20 Red Sindhi × Jersey (F_1) cows were examined (a) at a "normal" temperature of 57°–84° F., and (b) after 3 hours exposure to 105° F. with a vapor pressure of 34 mmHg. Observations were made on rectal temperature, respiratory rate, and respiratory volume. The method of measuring respiratory volume is described.

The crosses showed a smaller rise in rectal temperature (1.87 ± 0.152° F.) than did the Jerseys (3.06 ± 0.236° F.). Both the final respiratory volume and the rise in respiratory volume, however, were also slightly smaller in the crosses. Furthermore, the correlation between respiratory volume and rectal temperature in cows exposed to comparable heat stress was a positive one, often of high magnitude.

It is concluded that the superior heat tolerance of the crosses cannot be attributed to a more sensitive respiratory response, but that the higher respiratory activities are compensatory responses displayed by the individuals with the lower heat tolerance. The crosses, however, did show a more marked reduction of tidal volume (-0.48 ± 0.131 l./resp.) than the Jerseys (-0.29 ± 0.113 l./resp.), which should further reduce the risk of an induced alkalosis.

Respiratory rate proved to be a less sensitive index of heat tolerance than respiratory volume. Under these conditions there was no significant correlation between rectal temperature and respiratory rate.

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PERFORMANCE OF HOGS OF DIFFERENT BREEDS AND FROM STRAIGHTBRED AND CROSSBRED DAMS ON WISCONSIN FARMS [1]

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FEW comparisons of the prolificacy, viability, and rate of gain of farm-raised hogs of the different breeds have been made. Lush and Molln (1942), using records from college herds and from state and federal experiment station herds, compared eight breeds of hogs for litter size at birth and weaning and weaning weight of the litter. They found highly significant differences between the breeds for each of these characteristics. There are several publications on the effects of crossbreeding swine for market hog production, among them those by Carroll and Roberts (1942), Lush *et al.* (1940), Robison (1948), and Winters *et al.* (1935). The results of these experiments indicate a consistent though not large superiority of the crossbreds over the purebreds. The interpretation of these results, however, and the opinion as to the economic soundness of the practice when all factors are considered, vary.

Knowledge of differences between breeds and between purebreds and crossbreds is of interest not only to hog producers, but to investigators in genetics, nutrition, and other aspects of swine experimentation as well. This paper presents information on the performance of four pure breeds, and on the performance of purebred and of crossbred dams.

Materials and Methods

Records of pigs raised on Wisconsin farms have been made available to the University for research purposes through the Wisconsin Swine Selection Co-operative. The data on 2827 gilt litters farrowed in the years 1947 to 1950 inclusive were analyzed in this study; these litters represented 24,576 pigs born and 18,533 raised to the age of 5 months in 628 herds. The breed of the gilt farrowing the litter was known in all cases, and the breed of the sire was known for nearly four-fifths of the litters. Spotted Poland China, Duroc, Chester White, and Poland China breeds, and crossbreds, were repre-

[1] Paper No. 511 from the Department of Genetics and the Department of Animal Husbandry. Published with the approval of the Director of the Agricultural Experiment Station. This work was supported in part by a grant from Oscar Mayer and Company, Madison, Wisconsin.

sented. No other breeds were represented by more than a few litters and hence were not included.

Three separate analyses were made of the data. The first, based on 2189 litters in 512 herds, was of the differences in performance between the four straightbred breeds of dams with the breeding of the litter not considered. The second was of the differences in litter weights, 154-day pig weights, and pig indexes of the 1470 straightbred litters farrowed in 350 herds by these four breeds of gilts. Thirdly, a comparison was made of the effects of purebreeding and crossbreeding on gilt performance. Litters from the 2189 straightbred gilts were compared to the litters from 638 crossbred gilts, the crossbreds and straightbreds each being considered as a single group for this comparison. The number of crossbred pigs from any of the breeds of straightbred dams was too small to make a comparison of straightbred and crossbred litters from the same breed of dam.

Characteristics studied by analysis of variance were: (1) number farrowed; (2) number raised to 5 months; (3) litter weight at 5 months [1]; (4) sow productivity [2]; (5) average index [3]; (6) pig weight at 5 months.[4] The percent viability was not calculated for each litter, but was computed from the totals for numbers farrowed and raised per group. Chi-square analysis was used in studying this characteristic.

In practically all of the data in this survey, pigs of one breed or type of breeding in one group of herds were compared with pigs of another breed or type of breeding in another group of herds. Those few herds which had two or more breeds were considered as two or more herds for purposes of statistical analysis. Hence, herds as referred to herein are actually "breed-herds."

In the analysis of variance of litters from the four straightbred breeds of gilts, and of the straightbred groups of pigs, the variation was divided into the following components: between breeds, between years, interaction of breeds and years, between herds within breeds and years, and within herds. The mean square for between herds within breeds and years was tested for significance against the within herd mean square. The interaction mean square was tested against

[1] Divided by 50 for card punching.

[2] $N_f + 2N_w + \frac{\text{Weaning Wt. of Litter}}{15}$, in 1947 and 1948. In 1949 and 1950, no weaning weights were taken. Sow productivity was then computed as $N_f + 2N_{154} + \frac{\text{154-day Wt. of Litter}}{100}$ Ten points were added for gilt litters in both cases. The two estimates of sow productivity give essentially equal results.

[3] Individual pig index = sow productivity + 0.7 (wt. at 154 days − 74).

[4] Adjusted to 154-day equivalent when pigs were not weighed at exactly 154 days. The weight of one pig taken at random from each litter was used in this part of the study.

the between herd term, as were the between breed and between year mean squares where the interaction was not significant. Where there was a significant interaction between years and breeds, this mean square was used in testing for differences between breeds and between years.

The variation in purebred and crossbred dams was divided into the same components as above with the exception that the between herd mean square was within mating systems and years, rather than within breeds and years, and therefore contained any differences there were between breeds. Since this was not considered to be a logical error term to use in testing for differences between the two mating systems, the mean square for between herds within breeds and years from the comparison of the four straightbred breeds of dams was used in testing for significance of interaction between mating systems and years, and for significance of differences between mating systems and between years where there was no significant interaction between these two factors. The mean square from the previous analysis was used because no term for between herds within breeds for the pooled purebred and crossbred group was available.

In order to determine more specifically to what breed or group of breeds any significant differences found among the breed means were due, the method described by Tukey (1949) for comparing individual means from the analysis of variance was used.

The viability of each breed was compared individually with the viability of each other breed and straightbreds were compared with crossbreds by means of X^2. These tests were made on data pooled over all herds for each year.

The different breeds were not represented in exactly the same proportion in each year's data, nor was the percentage of crossbreds the same from each breed of gilt (table 1). The disproportion in numbers, however, was not considered sufficient to warrant attempting to correct for it.

Results

The numbers of litters in each breed group for each year are given in table 1. After the analyses of the records for 1947 to 1950, inclusive, were completed, the data for 1951 and 1952 became available and are included in tables 1, 2, and 3. The averages of all characteristics for these last 2 years for the different groups are so similar to those of the preceding years that it was considered unnecessary to reanalyze

the data but useful to present the averages based on the larger numbers of observations.

Mean squares derived from the analyses of variance of these data for 1947–1950, inclusive, are given in tables 4, 5, and 6. The four breeds of dams differed from each other in all litter characteristics studied, but differences between their pigs in individual weight at 5 months were not significant. Of the characteristics of the straightbred pigs which were analyzed, litter weight and average index showed significant differences, but again weight of the individual pigs did not.

TABLE 1. DISTRIBUTION OF LITTERS BY BREED FOR THE 6 YEARS 1947–1952

Year	Breeding of Litter	Breed of Dam						
		Spotted Poland China	Duroc	Chester White	Poland China	All Straightbred	All Crossbred	All Groups
1947	Straightbred	18	64	49	55	186	..	186
	Crossbred	21	18	10	15	64	93	157
	Unknown	15	75	48	90	228	..	228
1948	Straightbred	31	176	73	108	388	..	388
	Crossbred	13	7	6	14	40	139	179
	Unknown	0	28	16	16	60	..	60
1949	Straightbred	77	213	140	123	553	..	553
	Crossbred	28	11	8	6	53	213	266
	Unknown	0	39	13	11	63	..	63
1950	Straightbred	29	136	95	83	343	..	343
	Crossbred	28	38	15	7	88	193	281
	Unknown	35	35	36	17	123	...	123
1951	Straightbred	27	104	65	50	246	..	246
	Crossbred	6	10	20	5	41	71	112
	Unknown	23	42	32	12	109	..	109
1952	Straightbred	61	101	49	111	322	..	322
	Crossbred	21	21	4	0	46	127	173
	Unknown	1	28	14	9	52	..	52
All years	Straightbred	243	794	471	530	2038	..	2038
	Crossbred	117	105	63	47	332	836	1168
	Unknown	74	247	159	155	635	..	635
	Total	434	1146	693	732	3005	836	3841

Comparison of the individual means within each of the seven comparisons (five in table 4, two in table 5) which gave significant differences between breeds showed that the Poland Chinas were significantly inferior to each of the other three. When the three remaining breed means were compared, there was an indication (P=.05) that the Spotted Poland China gilts farrowed fewer pigs than did the Durocs and Chester Whites; however, Spotted Poland China gilts farrowing straightbred pigs had a litter size intermediate between

TABLE 2. NUMBERS AND WEIGHTS OF PIGS FARROWED AND RAISED BY STRAIGHTBRED GILTS OF DIFFERENT BREEDS AND BY CROSSBRED GILTS FOR THE YEARS 1947–1952 INCLUSIVE

Breed of Dam	Number of Litters	Number Farrowed	Number Raised to 154 Days	Viability	Litter Weight at 154 Days	Gilt Productivity	Average Index	Pig Weight at 154 Days
				%	lb. ÷ 50			lb.
Spotted Poland China	434	8.52	6.83	80.2	18.9	42.9	88.1	136.4
Duroc	1146	9.20	6.86	74.5	18.8	44.0	88.8	136.6
Chester White	693	8.90	6.68	75.0	18.2	43.0	86.5	135.9
Poland China	732	7.79	5.70	73.3	15.3	38.6	80.8	133.2
All straightbred	3005	8.69	6.53	75.2	17.8	42.3	86.2	135.6
All crossbred	836	8.92	7.20	80.8	19.6	44.9	89.0	135.8

TABLE 3. NUMBERS AND WEIGHTS OF STRAIGHTBRED PIGS FARROWED BY GILTS OF DIFFERENT BREEDS FOR THE YEARS 1947–1952 INCLUSIVE

Breed of Dam	Number of Litters	Number Farrowed	Number Raised to 154 Days	Viability	Litter Weight at 154 Days	Sow Productivity	Average Index	Pig Weight at 154 Days
				%	lb. ÷ 50			lb.
Spotted Poland China	243	8.70	6.92	80.0	19.2	43.2	88.3	139.0
Duroc	794	9.31	6.92	74.3	19.3	44.4	90.4	138.9
Chester White	471	8.78	6.56	74.7	17.9	42.5	86.2	136.1
Poland China	530	7.84	5.82	74.3	15.6	38.6	80.7	132.6
All breeds	2038	8.73	6.55	75.0	18.0	42.1	86.7	136.6

TABLE 4. MEAN SQUARES FROM ANALYSIS OF DIFFERENCES BETWEEN PERFORMANCES OF FOUR BREEDS OF GILTS

Source of Variation	Degrees of Freedom	Number Farrowed	Number at 154 Days	Litter Weight at 154 Days	Sow Productivity	Average Index	Pig Weight at 154 Days
				lb. ÷ 50			lb.
Between years	3	5.67	24.33*	421.7*	1406.0	1144.7	5056.3
Between breeds	3	243.0**	171.0**	1779.0**	3592.3*	10346.0**	3330.7
Interaction years and breeds	9	16.0*	13.77	195.7	703.8**	2443.9	3846.8
Between herds within breeds and years	496	8.06**	8.30**	132.8**	175.1**	1781.5**	2911.5**
Within herds	1677	4.23	4.21	33.4	75.6	211.4	579.4

* Significant at the 5% level.
** Significant at the 1% level.

these other two breeds. The other six comparisons of the three breed means did not indicate any heterogeneity in the group.

The size of litters from crossbred gilts was found to be significantly large than that from straightbred gilts at 5 months of age, though not at birth. Crossbred gilt litters were also superior in litter weight at 5 months and in sow productivity.

Chi-square tests of the differences in viabilities between the breeds, year by year, show the viability of the Spotted Poland Chinas to be significantly superior to each of the other breeds. No other clear-cut differences in viability were evident between any of the other three

TABLE 5. MEAN SQUARES FROM ANALYSIS OF DIFFERENCES BETWEEN STRAIGHTBRED PIGS OF FOUR BREEDS

Source of Variation	Degrees of Freedom	Litter Weight at 154 Days	Average Index	Pig Weight at 154 Days
		lb.÷50		lb.
Between years	3	152.7	685.3	150.7
Between breeds	3	1094.0*	8198.0**	4915.3
Interaction years and breeds	9	283.9*	2716.1	4073.7
Between herds within breeds and years	334	126.4**	1746.2**	2884.2**
Within herds	1120	32.6	189.1	536.9

* Significant at the 5% level.
** Significant at the 1% level.

breeds. Chi-square tests also showed consistently significant superior viability of pigs from crossbred dams compared to those from straightbred dams for each year.

Discussion

Lush and Molln (1942), in their report of the comparisons of sows of different breeds, gave figures for size of litter farrowed by Duroc, Chester White and Poland China gilts of 8.67, 8.38 and 7.02 pigs, respectively. The corresponding figures for the three breeds in this study are 9.31, 8.78 and 7.84, which, while they are somewhat higher than the former, rank the breeds in the same order. No figures for Spotted Poland Chinas were reported by these authors. The figures they gave for number weaned by gilts of these three breeds were 5.34, 5.56 and 4.78, while the present survey shows the corresponding figures, for number at 5 months, to be 6.92, 6.56, and 5.82. This is a viability for Durocs, Chester Whites and Poland Chinas of 61.6,

65.6 and 68.1 percent, respectively, for the first report, and 74.3, 74.7 and 74.3 for the present one.

Few comparisons of the different pure breeds for rate of gain to market weight have been made. Some figures for purebreds are given in comparisons of purebreds and crossbreds (Robison, 1948; Winters *et al.*, 1935), but these are values for an individual breed or for a pooled group of purebreds. With the large differences which exist in environment from one experiment or one survey to another, such individual means are probably not directly comparable to each other or to the values obtained in this study. The present survey indicates

TABLE 6. MEAN SQUARES FROM ANALYSIS OF DIFFERENCES IN PERFORMANCE BETWEEN STRAIGHTBRED AND CROSSBRED GILTS

Source of Variation	Degrees of Freedom	Number Farrowed	Number at 154 Days	Litter Weight at 154 Days	Sow Productivity	Average Index	Pig Weight at 154 Days
				lb. ÷ 50			lb.
Between years	3	17.33	35.33**	597.0**	1542.7**	1896.7	7516.7
Between mating systems	1	3.0	172.0**	758.0*	1706.0**	266.0	1107.0
Interaction years and mating systems	3	10.0	0.33	140.0	267.3	1810.3	2423.0
Between herds and breeds within mating systems and years	620	9.35**	8.97**	149.1**	202.0**	1994.7**	3200.5**
Between herds within breeds and years (from table 4)	496	8.06	8.30	132.8	175.1	1781.5	2911.5
Within herds	2199	4.03	4.00	31.7	71.7	196.3	565.6

* Significant at the 5% level.
** Significant at the 1% level.

no significant differences in five-month weight between pigs of different breeds, between pigs farrowed by different breeds of gilts, or between pigs from straightbred and from crossbred gilts.

Lush *et al.* (1940), Robison (1948) and Winters *et al.* (1935) report results of crossbreeding experiments from which they all conclude that this practice produces hogs which show superior viability, rate of gain (and in some cases economy of gain) and litter size, over purebreds. Carroll and Roberts (1942), on the other hand, in a review of the reports of a large number of crossbreeding experiments including some of the ones above, concluded that crossbreeding is not superior to purebreeding. They took as a criterion of superiority, however, crossbreds which exceeded the higher parent, rather than

crossbreds which exceeded the mean of the two parental breeds. Also, in choosing the higher parent, they selected the higher parent for each individual experiment, which would mean in some cases choosing animals of one breed and in other cases animals of another breed. Averaging the higher parents selected in this manner and setting this as a standard for the crossbreds to excel would require that the latter be superior to the best individuals picked from all groups or breeds. Comparing the same crossbreds to the parent breed which over all has the highest average may lead to an entirely different conclusion. The latter comparison would seem more logical, since a breed is a more realistic population than is a group of the highest individuals from several breeds. Dickerson (1952), using the data summarized by Carroll and Roberts, shows that the crossbreds in these experiments were superior to the purebreds in each of the characteristics studied except number farrowed. Since the superiority of crossbreds over purebreds in each character would be compounded in an estimate of total performance, an even greater advantage would be shown by this measure of the practical merit of crossbreeding.

Winters *et al.* (1935) have stressed the superiority of crossbred sows compared to purebred sows, particularly when the former are mated to boars of a third breed. He states that such a mating gives the offspring the benefit of being crossbred themselves and the benefit of the vigor of crossbred dams on which they are dependent from conception to weaning. Dickerson (1952) has pointed out that the characteristics most adversely affected by inbreeding are prolificacy and suckling ability of sows and viability of the pigs; crossbreeding, as would be expected, has the opposite effect, giving its greatest increase in these characters. Durham *et al.* (1952), in a comparison of inbred and non-inbred boars mated to outbred sows, found the greatest gains from topcrossing (which in some instances in this experiment was also crossbreeding) in the productivity of the gilts sired by the inbred boars. Litters weaned by topcross gilts were significantly heavier than those weaned by non-topcross gilts, and three-fourths of the increase was due to a larger number of pigs farrowed and a higher viability of these pigs combining to give larger litters weaned. Thus, there appears to be increasingly convincing evidence of the favorable effect of crossbreeding on sow productivity.

The results of the present survey add to this evidence. While size of litter farrowed and weight of the individual pigs were not significantly higher for crossbred than for straightbred dams, the increase in viability was substantial and contributed to a significantly higher

litter size and litter weight at 5 months, and higher sow productivity index.

Summary and Conclusions

Data on 2827 litters of pigs farrowed and raised by gilts on Wisconsin farms in the springs of 1947 to 1950 have been analyzed for differences between four breeds of dams in sow productivity and in 154-day individual pig weights. Similar analyses were made of records of straightbred pigs of these four breeds and of the performance of straightbred versus crossbred dams. The breeds considered were Spotted Poland China, Duroc, Chester White and Poland China.

A significant difference found between the breeds of dams on the litter characteristics studied was due almost entirely to a smaller litter size farrowed by the Poland China breed. It was also true that Spotted Poland China gilts farrowed pigs that had a lower mortality than did those of any of the other breeds.

Litters from crossbred dams had a lower mortality than litters from straightbred dams, but crossbred dams showed no other marked advantage.

No significant differences in five-month weight were found between any of the breed groups compared.

Data from the same source on an additional 1014 gilt litters in the two subsequent years (1951–1952), in which the four breeds and crossbreds were represented in about the same proportion as before, give approximately the same average values. These averages are presented in combination with the earlier data.

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AN EVALUATION OF SOME SWINE CARCASS MEASUREMENTS

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IN animal breeding investigations it is essential that the measures used to evaluate traits be as closely associated with the traits as possible. Random errors in trait appraisal act in the same manner as environmental variations in reducing the effective heritability of a desired trait. Consequently, it is necessary to be ever striving to find new and better measures of traits of economic importance. In the interests of economy it is also important to occasionally evaluate those measures in use and discard those that are of no practical value.

This paper presents the results of a study which was made on some measures of swine carcass merit. A companion paper (Whiteman *et al.*, 1953) reports the results of a similar study comparing carcass specific gravity and average back fat thickness as measures of carcass value.

Materials and Methods

The 203 carcasses used in this study were from the four farrowing seasons of 1949 and 1950. Of these, 183 pigs were principally of outbred, inbred, and line-cross Duroc, Duroc crossbred and Landrace Poland breeding from the Swine Breeding Project conducted by the Oklahoma Experiment Station in cooperation with the Regional Swine Breeding Laboratory. The other 20 pigs were from the Animal Husbandry swine herd and were about equally divided between the Chester White, Hampshire, Berkshire and Poland-China breeds.

The pigs were taken off feed for 24 hours when they reached the weight range of 219 to 232 pounds. The slaughtering was done in the college meat laboratory. Carcasses were prepared packer style with the head off and leaf fat removed. After thorough chilling, the entire carcasses were measured and cut in order to get the following and other weights and measures:

1. Specific gravity (Sg) was obtained by dividing the air weight of the carcass by the air weight minus the water weight (Whiteman *et al.*, 1953).

[1] Animal Husbandry Department in cooperation with the Regional Swine Breeding Laboratory (Ames, Iowa), Bureau of Animal Industry, Agricultural Research Administration, U.S.D.A.

2. The average back fat thickness (BF) was the average of three measures taken opposite the third and last ribs and the sixth lumbar vertebra.

3. The loin lean area (La) as indicated by the product of the length and width measurements of the loin eye muscle (cross section) taken from the right loin at the last rib.

4. The loin lean area (Lap) as indicated by a planimeter reading of a tracing of the same loin eye muscles mentioned above.

5. The ham lean area (Ha) as indicated by the product of the length and width measurements of the lean exposed when the ham was removed from the loin end. This cut was made across a point half way between the aitch bone and the posterior end of the sixth lumbar vertebra and perpendicular to the long axis of the ham.

6. The ham lean area (Hap) as indicated by a planimeter reading of a tracing of the lean in the same hams mentioned above.

7. The percentages (based on chilled carcass weight) of three lean cuts (LC), the ham (H), the loin (L), and the ham and loin (H&L).

The hams were skinned about two-thirds of the way to the shank and the fat was trimmed to less than one-fourth inch in thickness. The loins had all external fat removed except scraps at the blade and loin ends. The shoulders were trimmed New York style with all external fat removed about two-thirds of the way to the shank.

Simple intra-breed correlations were calculated between all of the measurements for each of the seasons. It was apparent from the magnitude of these correlations that in the seasons (1949 fall and 1950 fall) in which there were sex differences (balanced within breeding groups), the coefficients of correlation were much higher than in the other seasons in which only barrows were slaughtered. Using the z transformation (Snedecor, 1948) it was found that the differences were probably real. Consequently, the coefficients for the seasons which included sex differences were combined to give the correlation coefficients hereafter referred to as the A group. The other two season's data were combined to give the B group. The z transformation was used in combining these coefficients of correlation.

Results

Tables 1 and 2 show two specific comparisons of special interest. The first comparison is between the two methods of measuring the loin lean area. Table 1 shows the correlation coefficients between each of these measures and the other measures of carcass merit.

It will be noted that although the planimeter reading gives a slightly higher set of correlation coefficients, the difference is significant in only two comparisons. The Hotelling (1940) test was used here in testing the difference between the two correlation coefficients obtained when correlating two estimates of the same variable to another variable.

TABLE 1. CORRELATIONS BETWEEN EACH METHOD OF MEASURING THE LOIN LEAN AREA (La AND Lap) AND OTHER MEASURES OF CARCASS MERIT

Measure	Group	La	Lap
Sg	A	0.602	0.689*
	B	0.336	0.465
BF	A	—.436	—.433
	B	—.264	—.289
H	A	0.604	0.642
	B	0.468	0.471
L	A	0.618	0.668
	B	0.440	0.527
H&L	A	0.681	0.721
	B	0.514	0.590
LC	A	0.667	0.681
	B	0.464	0.562
Ha	A	0.484	0.497
	B	0.607	0.727*
Hap	A	0.628	0.668
	B	0.607	0.700
La	A	.....	0.878
	B	.....	0.796

* Probability of chance occurrence $<.05$, Hotelling's Test of Significance (1940).

Table 2 is a comparison of the two methods of measuring the ham lean area. In this instance the planimeter method was better in the seasons where sex was a factor (group A), but the increased associations were not as great where sex differences were not included. The reason for this is not known.

Discussion

The estimates of association with other measures of carcass leanness shown by the two measures of loin lean area indicate that there is not much difference in the relative values. Since the length by width

estimate is much more easily obtained, it is thought to be the most useful. In either case the loin must be cut in two to determine the size of the eye muscle, which is a disadvantage to the commercial use of this measure of carcass merit. In breeding studies, it should be remembered that the size of the loin eye muscle determines the real value of the pork loin to a large extent; therefore, its degree of association with carcass leanness should be considered in any breeding

TABLE 2. CORRELATIONS BETWEEN EACH METHOD OF MEASURING THE HAM LEAN AREA (Ha AND Hap) AND OTHER MEASURES OF CARCASS MERIT

Measure	Group	Ha	Hap
Sg	A	0.439	0.667**
	B	0.374	0.402
BF	A	—.267	—.438**
	B	—.404	—.389
H	A	0.416	0.611**
	B	0.537	0.554
L	A	0.498	0.624*
	B	0.366	0.491*
H&L	A	0.513	0.685**
	B	0.490	0.584
LC	A	0.538	0.706**
	B	0.533	0.602
La	A	0.484	0.628*
	B	0.607	0.609
Lap	A	0.497	0.668**
	B	0.727	0.700
Ha	A	.....	0.793
	B	.....	0.840

* Probability of chance occurrence $<.05$.
** Probability of chance occurrence $<.01$.

system aimed at improving carcasses from populations that are deficient in the size of the loin eye muscle.

Since loin lean area is so important in determining the value of the loin, its combination with a measure such as specific gravity might predict carcass leanness better than either taken alone. This possibility was studied by the multiple correlation technique described in Snedecor (1948) using percentage lean cuts as a criterion of carcass leanness. The inclusion of loin lean area (La) with specific gravity raised the predictability of percentage lean cuts from 75 percent

(the square of the simple correlation between specific gravity and percentage lean cuts, 0.868) to about 79 percent (the square of the multiple correlation coefficient, 0.887), using data from the A group of carcasses.

The data indicate that the planimeter method of measuring the ham lean area is better than the estimate obtained from the product of the two dimensional measurements. However, even the planimeter measurement is less closely associated with carcass leanness than is either specific gravity or average back fat thickness. Further, there is no carcass cut-out value that is dependent enough upon ham lean

TABLE 3. SIMPLE CORRELATIONS AMONG TEN CARCASS MEASUREMENTS ON TWO GROUPS OF CARCASSES

		BF	Ha	Hap	La	Lap	L	H	H&L	LC
Sg	A	−.746	0.439	0.667	0.602	0.689	0.789	0.806	0.888	0.868
	B	−.482	0.374	0.402	0.336	0.465	0.450	0.572	0.586	0.647
BF	A	.....	−.267	−.438	−.436	−.433	−.623	−.634	−.701	−.785
	B	.....	−.404	−.389	−.264	−.289	−.311	−.527	−.457	−.590
Ha	A	.....	.....	0.793	0.484	0.497	0.498	0.416	0.513	0.538
	B	.....	.....	0.840	0.607	0.727	0.366	0.537	0.490	0.533
Hap	A	.....	.....	.....	0.628	0.668	0.624	0.611	0.685	0.706
	B	.....	.....	.....	0.609	0.700	0.491	0.554	0.584	0.602
La	A	.....	.....	.....	.....	0.878	0.618	0.604	0.681	0.667
	B	.....	.....	.....	.....	0.796	0.440	0.468	0.514	0.464
Lap	A	.....	.....	.....	.....	.....	0.668	0.642	0.721	0.681
	B	.....	.....	.....	.....	.....	0.527	0.471	0.590	0.462
L	A	.....	.....	.....	.....	.....	.....	0.614	0.883	0.817
	B	.....	.....	.....	.....	.....	.....	0.555	0.913	0.738
H	A	.....	.....	.....	.....	.....	.....	.....	0.912	0.888
	B	.....	.....	.....	.....	.....	.....	.....	0.852	0.885
H&L	A	.....	.....	.....	.....	.....	.....	.....	.....	0.951
	B	.....	.....	.....	.....	.....	.....	.....	.....	0.904
If r equals:				0.30	0.40	0.50	0.60	0.70	0.80	0.90
s_r equals:				+0.097	0.088	0.078	0.066	0.052	0.036	0.019
				−0.103	0.096	0.087	0.075	0.061	0.043	0.023

area to warrant its inclusion in a selection program. In short, there seems to be no justification for its use if such measures as specific gravity, average back fat thickness or loin lean area are used.

Throughout this portion of this study it has been assumed that percentage lean cuts was the best measure of carcass leanness. This may not be true in every case. As Callow (1950) observed, there is a great variation in the amount of fat in the lean tissues of the body. If certain hogs have a tendency to deposit more of their fat in their muscular tissue, this will increase the percentage lean cuts of their carcasses even though the actual leanness will not be changed.

Table 3 shows all of the intra-breed correlation coefficients that were calculated. These are included for the benefit of those wishing

estimates of some of the relationships not reported in this paper or the companion paper (Whiteman *et al.*, 1953). It will be noted that these associations as a whole are not in disagreement with those found by other workers.

Summary

The carcasses from 203 hogs were used to evaluate two methods of measuring the loin eye muscle and the lean in the butt of the ham. These carcasses were predominantly from Duroc and Duroc crossbred breeding. The associations between the measures in question and other measures of carcass merit were used to determine the most desirable methods of carcass evaluation.

The method of approximating the size of the loin lean area by using the product of the length and width was found to be about as good as a planimeter measure of a tracing of the muscle cross-section and was much easier to obtain. The planimeter measure of the lean in the butt of the ham was better than the length by width approximation but adds little or no information about the carcass if the specific gravity, average back fat thickness or loin lean area are known.

A complete table of correlation coefficient estimates is included. The table shows the associations found when sex was a source of variation (Group A) and the associations estimated when only barrow carcasses were used (Group B).

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STUDIES ON THE PROTEIN REQUIREMENT OF SUCKLING PIGS

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A SURVEY of the literature on the nutrition of the pig during the suckling period reveals no specific attempt to evaluate the protein requirement at this early stage of growth.

Analyses of sow's milk show values of approximately 29 to 34 percent protein on a dry matter basis (Hughes and Hart, 1935; Newlander and Jones, 1935; Braude *et al.*, 1947; Bowland *et al.*, 1949; Heidebrecht *et al.*, 1951). Purified diets for suckling pigs varying in protein content from approximately 24 to 28 percent have been used successfully (Neumann *et al.*, 1948; Lehrer *et al.*, 1949; Sheffy *et al.*, 1952).

An assessment of the protein requirements for the pig during the suckling period would add materially to the limited knowledge concerning the nutritional requirements at this early stage in the life cycle. It would have particular application in providing a sounder basis for formulating diets of better nutritive balance for studying the requirements of suckling pigs for other essential nutrients and also in the development of practical sow-milk replacements. With this in mind, studies were undertaken to obtain information on the protein requirements of the pig during the first month of life. Diets containing isolated soybean protein ("Alpha" protein [3]) and casein were used in four separate trials.

Experimental

Two-day old Yorkshire litter mate pigs were fed simulated milk diets containing different levels of protein prepared essentially as described by Clark (1927) and Kastelic *et al.* (1950). The pigs were assigned into groups according to weight, litter and sex and the groups randomly allotted to the dietary treatments. The pigs were

[1] The authors gratefully acknowledge the assistance of a financial grant supporting these studies from the Western Condensing Co., Appleton, Wisconsin. Vitamin B_{12} was generously supplied by L. Michaud, Merck & Co., Newark, N. J., and lecithin by J. Eichberg, American Lecithin Co., Woodside, L. I. 77, N. Y. Dr. L. E. Lloyd assisted with the statistical analysis.

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[3] "Alpha" protein, Glidden Co., Chicago, Ill.

housed in individual screen-bottom cages which were kept in a heated room. Heat lamps provided additional warmth and helped to keep the pigs dry. Three times daily the pigs were individually fed from bottles equipped with lamb's nipples. Growth responses and efficiency of feed utilization were used as measures for comparing the levels of protein over a 28-day period. Nitrogen balances and plasma protein levels were also measured in some cases.

Results

Studies Using Isolated Soybean Protein

Isolated soybean protein, which had been washed to remove the residual sulfites, plus a supplement of DL-methionine, was used as

TABLE 1. DIETS CONTAINING ISOLATED SOYBEAN PROTEIN

Ingredients	Protein Content of Diets (Air-Dry Basis)			
	16.0	20.0	24.0	28.0
	%	%	%	%
"Alpha" protein	18.11	22.65	27.27	31.82
DL-methionine	0.34	0.41	0.48	0.55
Cerelose	47.46	42.85	38.16	33.54
Lard	24.63	24.63	24.63	24.63
Lecithin	1.49	1.49	1.49	1.49
Minerals [1]	7.97	7.97	7.97	7.97
Vitamins [1]	+	+	+	+

[1] See Sewell *et al.*, Jour. Nutr. 49:435, 1953 for formulations.

a source of protein in the first two trials. A protein conversion factor of $N \times 5.71$ was used in computing these levels on the assumption that "Alpha" protein is predominately glycinnin, the principle protein of soybeans. In trial 1 (table 1) diets containing 16, 20 and 24 percent of protein on an air-dry basis were studied. A 28 percent level was fed in trial 2 since 24 percent gave the fastest growth in the first test.

The results from trials 1 and 2 are shown in table 2. It is apparent that in both trials the pigs on the highest level of protein grew most rapidly and made the most efficient use of their feed as compared with the pigs on either of the lower levels. Approximately 25 percent more nitrogen was retained on the 24 percent protein diet than on the 16 percent. Plasma protein appeared to be somewhat higher on the 24 percent protein level, although there was little difference in this respect between the pigs on the 20 and 16 percent levels.

TABLE 2. AVERAGE DATA FOR ISOLATED SOYBEAN STUDIES

Item	Protein Content of Diets, Percent			
	16.0	20.0	24.0	28.0
	Trial 1			
Number of pigs	3	3	3	..
Initial weight, lb.	3.08	3.52	3.52	..
Final weight, lb.	8.80	10.56	12.76	..
Daily gain, lb.	0.21	0.25	0.34	..
Daily gain, % of init. wt.	6.71	7.18	9.50	..
Apparent digestibility of N, %	96.49	..	95.44	..
Apparent digest. N retained, %	73.71	..	66.09	..
Nitrogen retained daily, gm.	4.10	..	5.19	..
Plasma protein, %	5.37	5.27	5.74	..
Air-dry feed/lb. gain, lb.	1.59	1.38	1.19	..
	Trial 2			
Number of pigs	..	3	3	3
Initial weight, lb.	..	2.64	2.66	2.71
Final weight, lb.	..	6.07	7.83	9.20
Daily gain, lb.	..	0.12	0.19	0.23
Daily gain, % of init. wt.	..	4.64	6.94	8.50
Air-dry feed/lb. gain, lb.	..	2.57	1.80	1.48

In trial 2, the pigs were from a first-litter gilt which refused to accept them. As a result they received only limited amounts of colostrum. This fact and the relatively small initial weights possibly account for their slow rate of growth in comparison with the pigs in trial 1. Nevertheless, the relative responses obtained in this trial are in good agreement with the results of trial 1, and show that 28 percent of protein in the dry matter of the diet permits more rapid growth and greater efficiency of feed utilization than either 24 or 20 percent of protein. Considering the small numbers of animals which were

TABLE 3. DIETS CONTAINING PROTEIN FROM CASEIN

Ingredients	Protein Content of Diets (Air-Dry Basis)				
	16.0	20.0	24.0	28.0	32.0
	%	%	%	%	%
Crude casein	18.66	23.28	27.90	32.55	37.15
Cerelose	47.25	42.63	38.01	33.36	29.16
Lard	24.63	24.63	24.63	24.63	24.63
Lecithin	1.49	1.49	1.49	1.49	1.49
Minerals	7.97	7.97	7.97	7.97	7.97
Vitamins	+	+	+	+	+

employed, it was decided to repeat the tests. Therefore, two additional trials were conducted using casein as the protein source.

Studies Using Casein

The composition of the diets containing casein is presented in table 3. In trial 3 the levels of protein used were 16, 20, 24 and 28 percent (N×6.38), expressed as percentage of the air-dry matter. A 32 percent protein level was also used in trial 4. Feeding, housing and management of the pigs were as described previously.

Originally five pigs were placed on each of the diets in trial 3 (table 4). However, during the first few days of the experiment, a severe outbreak of transmissible gastro-enteritis was encountered. As a result, seven pigs from the various lots died. Prior to death the sick pigs showed abnormally high lucocyte counts and generally ran slightly elevated rectal temperatures. Diagnostic confirmation was obtained by transmission tests.[4] Inspection of the individual growth performance of the surviving pigs showed that they made consistent gains after recovery from the outbreak and, thus, the results are shown for any value they may have. The data (table 4) show that as the protein content of the diet was increased from 16 to 28 percent there was a progressive increase in weight gains of the pigs. This increase was accompanied by increased economy of feed utilization as shown by the units of air-dry feed intake required to produce a unit of gain. These results corroborate the previous data obtained in the studies involving isolated soybean protein.

This trial was repeated in order to give two trials on each type of protein and in addition to include a diet containing 32 percent of protein. The data (trial 4, table 4) show that the pigs receiving 32 percent of protein in their diet grew at a faster rate than any of the others, and they required less air-dry feed to make a pound of gain. One pig on the 28 percent protein diet died on the fifth day of the experiment from an undiagnosed cause. Two other pigs in this group failed to give consistent responses for no apparent reason. This accounts for the slower average rate of growth obtained on the 28 than on the 24 percent level. However, with the exception of that group there was a consistent increase in the rate of gain and feed efficiency for each increase in protein.

A statistical analysis of trial 4 revealed that the average daily gain

[4] Diagnosis by Dr. J. A. Baker, Veterinary Virus Research Institute, Ithaca, N. Y.

for the pigs fed 32 percent of protein was significantly greater (odds 19:1) than for the 16 or 20 percent, but not for the diet containing 24 percent of protein.

Discussion

Any attempt to evaluate the protein requirements of the pig at this early stage of development might logically be based on a consideration of the protein content of sow's milk. Numerous reports on the gross composition of sow's milk may be found in the literature.

TABLE 4. AVERAGE DATA FOR CASEIN STUDIES

Item	Protein Content of Diets, Percent				
	16.0	20.0	24.0	28.0	32.0
	Trial 3				
Number of pigs	4	3	3	3	..
Initial weight, lb.	2.99	2.90	2.86	3.04	..
Final weight, lb.	8.56	9.81	11.42	13.74	..
Daily gain, lb.	0.20	0.25	0.31	0.38	..
Daily gain, % of init. wt.	6.70	8.50	10.69	12.57	..
Air-dry feed/lb. gain, lb.	2.64	2.07	1.91	1.44	..
	Trial 4				
Number of pigs	4	4	4	3	4
Initial weight, lb.	4.09	4.00	4.09	4.04	4.07
Final weight, lb.	12.96	15.16	17.09	16.02	18.81
Daily gain, lb.	0.32	0.40	0.46	0.43	0.53
Daily gain, % of init. wt.	7.75	9.96	11.25	10.62	12.89
Air-dry feed/lb. gain, lb.	1.81	1.48	1.26	1.32	1.11

These are subject to wide variations, but in general the reported values for protein content range from approximately 29 to 34 percent on a dry matter basis. The fact that this concentration of protein is found in sow's milk might be expected to represent a possible upper limit which the requirement would not exceed. The experiments under discussion tend to support the view that a maximum rate of growth is possible only when the feed contains a protein content approaching the concentration found in sow's milk.

To illustrate more clearly the relation between protein content of the diet and rate of gain, the average data are shown in figure 1 by weekly periods. It is seen there were increases in weight gains as the protein level increased. In trial 4 the 32 percent level produced

more rapid growth than was obtained on the 24 or 28 percent protein diets. The data presented in figure 1 further suggest that the casein used provided a more satisfactory source of protein than did the isolated soybean protein supplemented with methionine as used in trials 1 and 2, since the casein diets resulted in slightly faster rates of growth. Adequate levels of vitamin B_{12} were supplied in crystalline form to all diets to insure that a lack of this vitamin would not retard growth.

Since the nutritional economy during this early stage of growth depends primarily upon a correct adjustment between the proportion of protein and the total energy supplied, it was thought of interest to examine the nutritive ratios of the various diets. The ratios between the total crude protein and the total calories were calculated to be 1:6.5, 1:5.1, 1:4.0, 1:3.3 and 1:2.9 for the diets containing 16, 20, 24, 28 and 32 percent of protein, respectively. In our tests most efficient and rapid gains occurred on diets with a nutritive ratio approaching 1:3. This is in accord with the early work of Washburn and Jones (1916) who suggested a nutritive ratio for growth in young pigs of 1:3. The nutritive ratios of sow's milk as calculated from reported values were between 1:3 and 1:4.

Although fastest growth rates were obtained in these tests on a diet containing 32 percent of protein, it is likely that often it may be more economical to feed simulated milks of lower protein content. In fact, this might be generally true if the young pig is able to fully recover from a slight, temporary retardation in growth rate following refeeding on adequate diets. To obtain information on this question, the pigs used in trial 4 were studied for 3 weeks after the end of the trial. The pigs were divided into three pens according to size, and they were all fed an adequate ration *ad libitum*. During the 3-week period the average daily gains were 1.19, 1.24, 1.10, 1.04 and 0.94 pounds per day respectively for the animals that previously received 32, 28, 24, 20 and 16 percent of protein in their diets. The retarded pigs continued to grow at the lowest rate, showing that recovery in size did not occur by the time usual weaning age was reached. In view of these results and the report of Catron *et al.* (1951) showing that 14 percent of protein is adequate for weanling pigs, it is clear that further studies are needed to define the protein needs of pigs during the transition period between 30 days and usual weaning age.

Summary

Four trials are reported in which isolated soybean protein and casein were used in simulated milk diets to study the protein requirements of baby pigs during the period from 2 to 30 days of age. Diets containing 16, 20, 24, 28 and 32 percent of protein, on an air-dry feed basis, were studied. The diets with only 16 or 20 percent of protein always gave slowest growth rates and resulted in retarded animals. Fastest growth and most efficient feed utilization occurred on the 32 percent protein diet, but 24 and 28 percent of protein gave relatively satisfactory results.

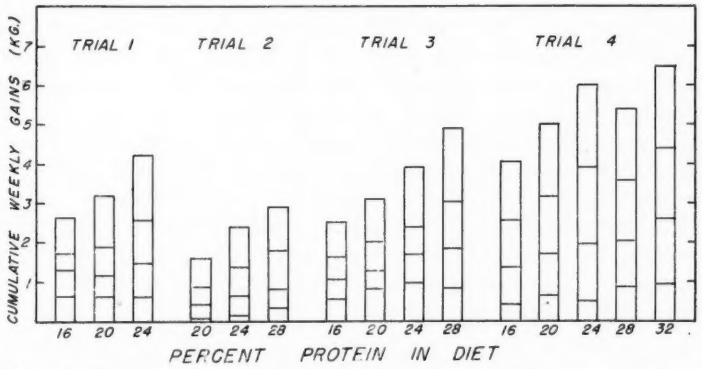

Figure 1. Showing the cumulative gains in weight by weekly periods of the various groups of pigs.

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THE SUPPLEMENTARY EFFECTS OF CALCIUM PANTOTHENATE AND AUREOMYCIN IN A LOW PROTEIN RATION FOR WEANING PIGS[1]

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THE general use of antibiotics in swine feeds has raised several questions concerning the effects of these substances on the requirement of the growing pig for dietary essential nutrients. A sparing effect of aureomycin, penicillin and streptomycin on the requirement of the rat for thiamine, riboflavin, pantothenic acid and pyridoxine has been found by Linkswiler *et al.* (1951), Lih and Baumann (1951) and Sauberlich (1951). In each of these cases the addition of one of the antibiotics to a diet containing limiting amounts of the particular vitamin being studied resulted in a significant growth response. Variations were noted in the relative effectiveness of different antibiotics towards deficiencies of a specific vitamin. However, aureomycin seemed to be particularly effective in pantothenic acid deficiency (Lih and Baumann, 1951). Biely and March (1951) found that aureomycin stimulated the growth of chicks fed diets containing suboptimum levels of niacin, folic acid or riboflavin. Stokstad and Jukes (1950) have found that aureomycin exerted a sparing effect on the vitamin B_{12} requirement of the chick and turkey poult.

Pantothenic acid deficiency can be produced in pigs fed certain rations made up principally of corn and soybean oil meal (Luecke *et al.*, 1949, 1950, 1952). Thus it seemed to be of practical importance to determine whether aureomycin would stimulate the growth of pigs fed a ration containing suboptimum levels of this vitamin.

Experimental

Sixty pasture raised cross-bred pigs approximately 6 weeks of age were divided uniformly into six experimental lots. The animals were sprayed, wormed and vaccinated for hog cholera at the start of the trial, and were self-fed the various rations in pens with concrete floors.

The basal ration was made up of the following ingredients (in per-

[1] Published with the approval of the Director of the Michigan Agricultural Experiment Station as Journal Article No. 1488.

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cent): corn, 81.8; soybean oil meal, 13.0; condensed fish solubles, 2.0; limestone, 1.4; dicalcium phosphate, 1.0; iodized salt, 0.7; trace mineral premix, 0.1. In addition, the following vitamins were added per pound of basal ration: niacin, 9 mg.; riboflavin, 2 mg.; thiamine, 1.5 mg.; pyridoxine·HCl, 1.5 mg.; vitamin B_{12}, 3 mcg.; vitamin A, 1500 I.U.; and vitamin D_2, 250 I.U.

The crude protein content of the basal ration was 15.4 percent, and the pantothenic acid content was 4.15 mg. per pound by micro-

TABLE 1. RESPONSE OF THE PIGS TO THE VARIOUS EXPERIMENTAL RATIONS

(Ten Pigs per Lot on Trials Lasting 10 Weeks)

Lot No.	Supplement	Av. Initial Wt.	Av. Daily Gain	Av. Daily Feed	Feed per Lb. Gain
		lb.	lb.	lb.	lb.
1	None [1]	25.1	1.15	3.57	3.10
2	Aureo. 5 mg./lb.[1]	25.1	1.24	3.89	3.14
3	Ca. pan. 2 mg./lb.	25.2	1.35*	4.00	2.96
4	Aureo. 5 mg./lb. & Ca. pan. 2 mg./lb.	25.1	1.58*	4.70	2.97
5	Ca. pan. 4 mg./lb.	25.0	1.38*	4.11	2.98
6	Aureo. 5 mg./lb. & Ca. pan. 4 mg. lb.	25.0	1.43*	4.18	2.92

[1] Five of the 10 pigs in each of lots 1 and 2 developed typical symptoms of pantothenic acid deficiency as characterized by locomotor incoordination.
* Daily gains significant over lot 1 ($P=0.05$).

biological analyses. The samples of feedstuffs were prepared for the pantothenic acid assay by the method of Buskirk *et al.* (1948). This method was found to give results comparable to those obtained using the pigeon liver enzyme and intestinal phosphatase mixture.

Results and Discussion

The results of the experiment together with the various experimental treatments are shown in table 1. Intermittent scouring was noted in some of the pigs in lots 1 and 2 by the fifth week of the trial. At the end of the eighth week, five of the 10 pigs in lot 1 showed typical symptoms of pantothenic acid deficiency as evidenced by locomotor incoordination. The remaining five pigs in lot 1 gained normally and at no time showed any evidence of deficiency.

The addition of crystalline aureomycin to the basal ration in lot 2 did not increase significantly the average daily gains. However, as in lot 1, five of the 10 pigs in this lot developed severe locomotor

incoordination. The deficiency symptoms in the five pigs in lot 2 appeared earlier and were more severe than in the deficient pigs of lot 1. The remaining five pigs in lot 2 were normal in every respect. A typical example of a deficient pig with locomotor incoordination is shown in figure 1.

Pigs in lot 3 (table 1) fed the calcium pantothenate-supplemented basal ration grew at a normal rate and no evidence of deficiency was observed. Inasmuch as the pantothenic acid content of the ration fed to the pigs in lot 3 was approximately 6.15 mg. per pound,

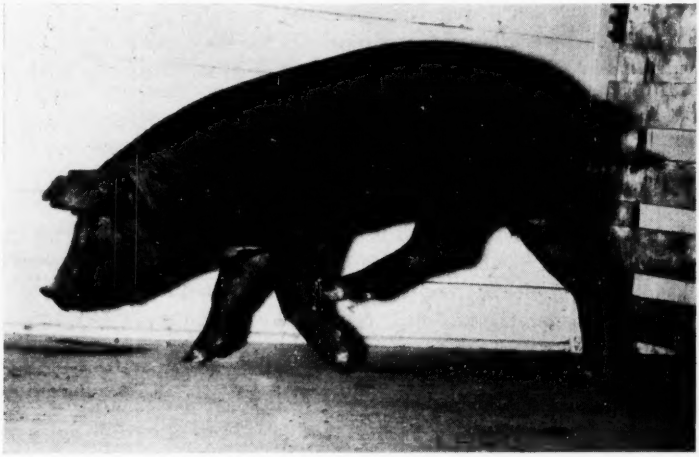

Figure 1. Representative pantothenic acid deficient pig from lot 2 showing locomotor incoordination typical of this deficiency.

it would seem to indicate that the requirement of the pig for this vitamin is certainly no higher than this level.

The addition of aureomycin and calcium pantothenate to the basal ration in lot 4 (table 1) resulted in excellent growth especially when one considers the low protein content of the ration and the low initial weight of the pigs. No evidence of deficiency was observed in this lot.

The addition of 4 mg. per pound of calcium pantothenate to the basal ration (lot 5) did not result in any increase in daily gains over that made by the pigs fed the 2 mg. per pound supplementary level.

The pigs in lot 6 fed both aureomycin and the higher level of calcium pantothenate did not gain quite as rapidly as the pigs in lot 4

fed the antibiotic and the lower level of calcium pantothenate. However, this difference was not statistically significant, and it would seem to be very unlikely that any antagonism between the vitamin and antibiotic occurred.

Inasmuch as one-half of the pigs in both lots 1 and 2 developed pantothenic acid deficiency while the remaining animals were normal, the calculation of the average daily gains for each lot as shown in table 1 is misleading. Accordingly, in an attempt to more clearly indicate the true effects of pantothenic acid deficiency on growth, the average daily gains for the five normal and five deficient pigs in each of lots 1 and 2 were calculated and presented in table 2. Since the animals in each lot were group-fed, the same calculations could not be made for feed intake. It can be seen from table 2 that the average

TABLE 2. AVERAGE DAILY GAINS OF PIGS FROM LOTS 1 AND 2 WITH AND WITHOUT PANTOTHENIC ACID DEFICIENCY SYMPTOMS [1]

Lot No.	Average Daily Gain	
	Deficient	Normal
	lb.	lb.
1	0.98	1.31**
2	0.99	1.49**

[1] Average daily gains for five pigs.
** Average daily gains of normal pigs in lots 1 and 2 highly significant over average daily gains of deficient pigs in lots 1 and 2 ($P=0.01$).

daily gain of 1.31 lb. for the normal pigs in lot 1 was approximately equal to the average daily gain of the pigs in lot 3 fed supplemental calcium pantothenate. Furthermore, the average daily gain of the normal pigs in lot 2 was also of the same order of magnitude as the average daily gains of pigs in lots 4 or 6 fed both the aureomycin and calcium pantothenate.

While it is probably fortuitous that one-half of the animals in each of lots 1 and 2 developed deficiency symptoms, the evidence does not indicate any sparing action of aureomycin on the pantothenic acid requirement. These results are at variance with the results previously mentioned obtained by other workers with rats. However, it should be mentioned that in the work with rats, levels of aureomycin of from 25 to 45 mg. per pound of diet were used. Thus it is possible that if these higher levels of aureomycin had been used

in the present trial a sparing action might have been observed. The supplementary level of 5 mg. of aureomycin per pound of feed was selected for the present trial because it represents a practical addition under common swine feeding conditions.

A point often overlooked in attempting to evaluate quantitative requirements of the pig for specific nutrients such as vitamins is the apparent wide variation in requirements between individual animals. This point is clearly brought out in the present experiment in the results obtained in lots 1 and 2. For one-half of the pigs in each of these lots a level of 4.15 mg. of pantothenic acid was sufficient for normal growth, while for the remaining half it was entirely inadequate. Whether these variations observed were due to differences in amounts of pantothenic acid required by each animal to carry on normal metabolic processes or were due to differences in the ability of each animal to utilize the vitamin as it occurred in the ration is not known.

While the present experiment indicates that the pantothenic acid requirement of the pig is greater than 4.15 mg. but less than 6.15 mg. per pound of feed, it must be emphasized that the results are applicable only for the feed ingredients used in the present trial. Certainly, one must consider that while the microbiological assay for any vitamin may reveal the total amount present in the feed, it does not necessarily indicate its physiological availability. Thus the physiological availability of pantothenic acid as it occurs in commonly used feed ingredients may vary widely, and this variability would not be revealed by the conventional microbiological assay. Thus when one considers these probable differences in physiological availability together with apparent differences in pantothenic acid requirement between individual pigs, the difficulties in attempting to set a certain figure as representing the pantothenic acid requirement of the pig are obvious.

Summary

Sixty 25-pound pigs were used to study the effect of aureomycin on the pantothenic acid requirement. A low protein basal ration (15.4 percent) containing 4.15 mg. of pantothenic acid per pound was used.

Five out of 10 pigs fed the basal ration developed symptoms of locomotor incoordination. When aureomycin was added (5 mg. per pound) to the basal ration, an equal number of pigs developed deficiency symptoms indicating no sparing action on the part of this antibiotic for pantothenic acid. However, the addition of calcium

pantothenate at a level of 2 mg. per pound to the basal ration prevented all symptoms of deficiency from appearing.

Possible reasons for apparent variations in pantothenic acid requirement of pigs are discussed.

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ABSORPTION AND UTILIZATION OF RADIOACTIVE CHARCOAL BY SWINE[1]

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SEVERAL feeding trials have been conducted to determine the value of wood charcoal and related materials as supplements to swine rations. Evvard *et al.* (1927), Thompson (1932) and Frolich and Luthge (1934) reported a growth stimulation resulting from charcoal additions to some common swine rations. Willman and Morrison (1947) using balanced natural rations and workers at this station (unpublished data) using well balanced rations, both natural and synthetic, found no beneficial effects either in growth rate or feed efficiency from additions of charcoal to the basal rations. No evidence has been presented to show whether charcoal is broken down in the gastrointestinal tract and utilized or if it passes through the animal in an inert form. If no absorption occurs, it would be of questionable nutritional value for swine.

This problem suggested the application of a balance study with swine using radioactive charcoal as the test material. The data submitted in this report were obtained by such a study in which the radioactive charcoal was produced by neutron bombardment of the charcoal in a pile reactor.

Experimental Procedure

A partial chemical analysis of the charcoals used is shown in table 1. It is noted that both hardwood and softwood charcoals were used. The hardwood No. 36 grade product was the one which had been employed in the feeding trials conducted at this station. It had been carbonized primarily from oak wood and had a texture resembling granulated sugar.

The softwood No. 80 grade product was used to facilitate a comparison between charcoals from hard and soft woods. It had been

[1] The data in this paper are taken from a thesis submitted by the senior author to the Graduate School of Purdue University in partial fulfillment of the requirements for the Ph.D. degree.

[2] The authors wish to express their appreciation to the National Mineral Feeds Association, Inc., 212 Equitable Bldg., Des Moines 9, Iowa, for establishing a grant to support this project.

[3] Contribution from the Department of Animal Husbandry, Journal Paper No. 672, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

carbonized from resinous pine wood by desructive distillation, and was of much finer texture than the hardwood No. 36 product. Following a 30-day period of irradiation in the pile reactor at Oak Ridge, Tennessee, the two charcoals arrived at the laboratory on May 2, 1952. As each product contained considerable activity, which exhibited a short half-life, a period of 8 days was allowed to elapse before administration of each product.

Each type of irradiated charcoal was administered orally to two pigs using a modification of the technique described by Hansard *et al.* (1951b). After a stomach tube was inserted into the stomach, a short-stemmed glass funnel was inserted in the upper end of the tube.

TABLE 1. A PARTIAL CHEMICAL ANALYSIS OF THE PRINCIPAL INORGANIC ELEMENTS IN THE TWO CHARCOALS USED IN THIS STUDY

	Type of Charcoal			
	Hardwood No. 36 (11.8% Ash)		Softwood No. 80 (9.6% Ash)	
Inorganic Element	Charcoal Basis [1]	Ash Basis	Charcoal Basis [1]	Ash Basis [2]
Calcium, %	5.24	44.41	5.20	54.17
Phosphorus, %	0.034	0.29	0.049	0.51
Manganese, %	0.102	0.86	0.185	1.93
Iron, %	0.047	0.40	0.044	0.46
Copper, %	0.0084	0.07	0.0074	0.08
Cobalt, p.p.m.	0.35	2.97	9.0	93.8

[1] These values are expressed as a proportion of the total charcoal.
[2] These values are expressed as a proportion of the total ash.

The charcoal, mixed with water in a beaker, was then poured into the funnel. Adhering charcoal particles were washed down with a 4-ounce sheep dosing syringe from which water was forced through the tube under pressure. No charcoal or radioactivity was detected in the beaker or stomach tube after administration.

Standards of each charcoal were prepared by placing a weighed amount into a 100 ml. volumetric flask and making to volume with a weak solution of hydrochloric acid.

From the time of administration to sacrifice, the animals were maintained in a metabolism stall where quantitative separate collections of feces and urine were made twice daily according to the methods described by Hansard *et al.* (1951c). They were fed a typical adequate ration *ad libitum* and were watered three times daily during this period.

After each pair of pigs was sacrificed, the urine, feces and several tissue samples were analyzed in duplicate for total radioactivity. Twenty-five milliliter aliquots of urine were concentrated to 10 ml. under infra-red heat lamps. One milliliter aliquots were placed in three-fourths inch diameter glass counting cups, dried and counted for 4 minutes with a thin window geiger tube. Samples of feces and

TABLE 2. DESCRIPTION, AMOUNT ADMINISTERED AND TIME SCHEDULE OF THE ORAL ADMINISTRATION OF IRRADIATED WOOD CHARCOAL TO PIGS

Items Compared	Pig 1 [1]	Pig 2 [1]	Pig 3 [2]	Pig 4 [2]
Length of adjustment period, hr.[3]	72	72	24	24
Time from administration to sacrifice, hr.	120	120	96	96
Description of irradiated charcoal	Softwood No. 80 grade	Hardwood No. 36 grade	Softwood No. 80 grade	Hardwood No. 36 grade
Amount of charcoal administered, gm.	2.15	4.20	9.37	6.47
Total activity administered, counts per minute	7.1×10^7	11.0×10^7	30.2×10^7	16.8×10^7

[1] No. 1 and No. 2 were litter-mate pigs and were on experiment concurrently.
[2] No. 3 and No. 4 were litter-mate pigs and were on experiment concurrently, 13 days later than pigs 1 and 2.
[3] This period represents the time the pigs were confined to the metabolism unit prior to the administration of irradiated charcoal.

soft tissues weighing 15 to 20 grams and bone samples weighing about 5 grams were taken and wet ashed to a small volume with nitric acid and hydrogen peroxide. They were then made up to a volume of 15 to 25 ml. with distilled water, and 1 ml. aliquots were dried and counted as described for the urine.

Two pairs of healthy littermate pigs, approximately 4½ months old and each weighing very close to 150 pounds, were used. Table 2 indicates the time schedule of adjustment, administration of charcoal and sacrifice which was followed, and also gives the amounts and kinds of irradiated charcoal administered to each of the four pigs. The activity of the irradiated charcoal was not assayed, due to the difficulty and expense of characterizing an unknown mixture of isotopes. Total radioactivity administered to each pig (table 2) was an estimate based on the assumption that the efficiency of the counting

apparatus was 10 percent. In other words, the total counts per minute of the administered charcoal as recorded by the counting procedure were one-tenth of the values given in table 1. The first pair of pigs was allowed a 72-hour adjustment period before the administration of irradiated charcoal and sacrificed after a collection period of 120 hours. The second pair of pigs became adjusted very rapidly to the close confinement of the metabolism unit and consequently was allowed an adjustment period of only 24 hours before the administration of the irradiated charcoal. The collection period for the second pair was also shortened to 96 hours, since almost no activity had been detected in the feces on the fifth day in the case of the first pair of pigs.

The following methods were used in the chemical analyses of both the hardwood No. 36 and the softwood No. 80 charcoals. Calcium and manganese were determined by methods outlined by the Association of Official Agricultural Chemists (1945), phopshorus by a modification of the method of Fiske and Subbarow (1925), iron by the method of Jackson (1938), copper by the method of Hoste (1950) and cobalt by a modified combination of the methods of Kidson and Askew (1940) and Sandell (1944).

Results and Discussion

The rate of gain and feed efficiency of three of the pigs were good, while one pig, No. 3, lost weight during confinement to the metabolism unit. This loss of weight was possibly due to a fractured femur which was discovered at the time of slaughter and which probably occurred when the pig was being placed in the metabolism unit. The appetite and consistency of the feces of Pig 3 were apparently normal during the period of confinement to the metabolism unit, so the data obtained on this pig have been included with that of the other three pigs in spite of the loss in weght.

The amount of radioactivity recovered in the feces is given in table 3 for each pig by 24-hour periods. Considerable variation occurred among the four pigs. Pigs 1 and 2, which were on experiment at the same time, excreted about 15 percent more of the total administered dose than Pigs 3 and 4. This difference in the amount of radioactivity recovered was probably not caused by the shorter collection period for Pigs 3 and 4 since Pigs 1 and 2 had excreted very little activity during the fifth 24-hour period, and Pigs 3 and 4 did not excrete large

quantities of activity in their final 24-hour collection period. Within each pair the pig receiving hardwood No. 36 charcoal (Pigs 2 and 4) excreted a larger amount of radioactivity than those receiving softwood No. 80 charcoal, indicating a higher utilization of the softwood No. 80 product. The difference in favor of Pig 2 over Pig 1 was much greater than that of Pig 4 over Pig 3. On an average the pigs receiving hardwood No. 36 charcoal excreted almost 11 percent more of the administered radioactivity than the pigs receiving softwood No. 80 charcoal. It is not known if this difference was due to the differences in type or in physical form of the two charcoals. Considerng all four pigs, the average percent of ingested radioactivity which was accounted for in the feces was 58.04 percent. During the periods of peak radio-

TABLE 3. FECAL EXCRETION OF RADIOACTIVITY FOLLOWING ORAL ADMINISTRATION OF IRRADIATED WOOD CHARCOAL TO PIGS

Time After Oral Administration	Percentage of Total Administered Radioactivity			
	Pig 1	Pig 2	Pig 3	Pig 4
hr.	%	%	%	%
0–24	0.49	40.77	29.69	0.18
24–48	0.52	18.52	16.74	5.08
48–72	54.67	12.21	0.83	44.48
72–96	2.10	0.58	0.26	4.36
96–120	0.56	0.14	....	....
Total percent recovered in feces	58.34	72.22	47.52	54.10

activity excretion, counts ran as high as 8,537 counts per minute per gram of fresh feces. Visual observations revealed that much of the charcoal present in the feces was in about the same physical form as was the administered charcoal.

Considerable error was probably involved in the recovery of radioactivity in the feces. Beginning 14 days after the charcoal samples were removed from the reactor, standards were prepared for half-life determinations and for characterization of the energy of the radioactivity present in the charcoal. The range of the emitted rays in aluminum was determined. From a comparison of the decay and range curves thus obtained with those appearing in the literature, it was concluded that almost all of the activity present in the charcoal at the time the animals were sacrificed was due to calcium and phosphorus. Furthermore, it was estimated that approximately 70 percent

of the activity was due to calcium and 30 percent to phosphorus. Since calcium, a soft beta emitter, accounted for such a large proportion of the activity, and since the samples as prepared for counting were rather thick due to incomplete oxidation of organic material, it can be reasonably assumed that the actual quantities of radioactivity in the feces were considerably greater than the amounts determined due to self absorption.

The pattern of radioactivity excretion also showed considerable variation, and no consistent differences correlated with either type of charcoal or pair were noted. Most of the total radioactivity excreted by Pigs 1 and 4 was recovered in the feces during the third 24-hour period. Pigs 2 and 3, on the other hand, excreted the largest amount in the first 24 hours after administration, then decreasing amounts during subsequent 24-hour periods. Negligible concentrations of radioactivity in the wall of the small intestine and in the contents of the small intestine (net counts below background) indicate that almost all of the administered activity had been either absorbed or excreted by the time the pigs were sacrificed.

Only questionable amounts of radioactivity were detected in the urine. The net counts of most of the samples were below or only slightly above background. However, the consistent detection of even low counts probably indicated the presence of minute quantities of activity in the urine.

The soft tissues which were sampled (liver, kidney, heart, blood and muscle) consistently produced low net counts, which indicated the presence of traces of radioactivity. However, since all of the samples had a net count of less than background, the amount present could not be accurately quantitated. Higher counts were obtained for the liver than for other soft tissues in the case of each pig.

Bone samples contained an average of 20 times greater concentration of radioactivity than soft tissues, and most of the samples counted at least 2–3 times more than background. Concentrations of radioactivity in bone tissues are presented in table 4. With the exception of the femur samples of Pigs 3 and 4, the epiphyseal region of the bone contained larger concentrations of activity than the shaft region, an average of 167 percent more.

The general pattern of the distribution of radioactivity in the bones and soft tissues as described above is in close agreement with the distribution of calcium as reported by Hansard *et al.* (1952) for cattle, by Hansard *et al.* (1951a), Carlsson (1951) and Harrison and Harri-

son (1949, 1950) for the rat, and with the distribution of phosphorus as reported for the rat by Hevesy (1939, 1940). This could be considered as an additional indication that the radioactivity from the charcoal was due primarily to calcium and phosphorus.

By using average figures for the percentage of bone (Turman, 1952) in the bodies of swine, estimates of total radioactivity in those tissues were calculated. Since only samples of epiphyseal and shaft bones were obtained, it was not possible to calculate an average value for the

TABLE 4. BONE CONCENTRATION OF RADIOACTIVITY FOLLOWING ORAL ADMINISTRATION OF IRRADIATED WOOD CHARCOAL TO PIGS

Bone	Percentage of Total Administered Radioactivity per Gram of Fresh Tissue			
	Pig 1	Pig 2	Pig 3	Pig 4
Femur epiphysis	0.00170	0.00046	0.00036	0.00030
Femur shaft	0.00102	0.00038	0.00057	0.00043
Rib epiphysis	0.00348	0.00182	0.00235	0.00278
Rib shaft	0.00148	.....[1]	0.00157	0.00042

[1] The samples of rib shaft from Pig 2 were accidently lost during digestion.

total radioactivity in the total bone tissue of each pig. Therefore, only the lowest and highest concentrations of activity in the case of each pig were used, which yield the extremes of the possible total radioactivity in the bones. The true value probably lies somewhere between the two extremes, which were as follows for Pig 1 through 4 respectively (percentage of total administered radioactivity in the total bone tissue): 7.36 and 25.12; 2.85 and 13.63; 2.40 and 15.68; 2.25 and 20.82. These values show that appreciable quantities of radioactivity were present in the bones following the oral administration of irradiated charcoal.

Summary

A study was made of the behavior of radioactive wood charcoal following oral administration to growing-fattening swine. The data obtained, although not exact, show that certain mineral elements in hardwood and softwood charcoals were absorbed from the digestive tract and utilized for body function.

At least 58 percent of the total administered activity was excreted in the feces within 120 hours.

Only traces of activity were detected in the urine.

Readily detectable levels of radioactivity were deposited in the bones. Approximately two-thirds of this activity resulted from radioactive calcium and one-third from radioactive phosphorus.

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THE EFFECT OF SURFACTANTS ON THE GROWTH RATE OF SWINE[1]

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SURFACTANTS have been reported as growth stimulators for poultry (Ely, 1951; Ely and Schott, 1952) and swine (Luecke *et al.*, 1952). Predominant among those tested for chicks was a polyethylene oxide condensate ester of coconut fatty acids, termed lauryl ethylene oxide condensate. In the swine work reported, Ethomid C-15, an N-substituted fatty acid amide with the substituents being polyoxyethylene groups, was employed.

Surfactants ordinarily are either neutral or alkaline salts, or combinations of the two. Actually there are a hundred, or more, surface-active agents. Since their development is still in the experimental stage, it seemed worth while to learn more of the potential of these products as growth stimulators.

Experimental

Trial I

Weanling Duroc pigs, averaging 34 pounds, were divided into two pens. Lot I served as the control and received a basal ration composed of ground yellow corn 68.10%, soybean oil meal 26.35%, dehydrated alfalfa meal 5.00%, vitamin A and D oil, vitamin B_{12}, riboflavin, niacin and calcium pantothenate. A mineral mixture composed of steamed bone meal 40, ground limestone 40, and salt 20 was provided free choice. Lot II received 0.10% Ethomid C-15 in addition to the basal ration listed for Lot I. The protein of the basal ration was 18% and was decreased as the pigs became larger.

Trial II

Weanling Duroc pigs, averaging 35 pounds, were divided into eight lots and received a complete basal ration whose composition follows: ground yellow corn 72.15%, soybean oil meal 10.4%, fish meal 5.2%, meat scraps 5.2%, cottonseed meal 2.6%, alfalfa meal 2.6%, steamed bone meal, ground limestone, salt, vitamin A and D concentrate,

[1] Contribution from the Department of Animal Husbandry, Journal Paper No. 672, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

vitamin B_{12}, riboflavin, niacin and calcium pantothenate. Lot I served as the control and received only the basal ration. Lot II received the basal ration plus 7.5 mg. aureomycin per pound of total ration. Lots III, IV and V received 0.025%, 0.05% and 0.10%, respectively, of Ethomid C-15 in the basal ration. Lots VI, VII and VIII received 0.10%, 0.20% and 0.40%, respectively, of alkyl benbene sulfonate in the basal ration. As in Trial I, the protein content of the basal ration was lowered as the pigs became larger.

Results

Trial I

The incorporation of 0.10 percent of Ethomid C-15 into an all-plant protein, vitamin-fortified, basal ration for Duroc pigs, starting at an average weaning weight of 34 pounds, had no effect on growth rate, feed consumption or feed efficiency over a 115-day dry lot feeding period.

TABLE 1. THE EFFECT OF ETHOMID C-15 ON THE GROWTH RATE OF WEANLING PIGS

(Length of Trial—115 Days)

Category of Interest	Lot I Control	Lot II 0.1% Ethomid C-15
Number of pigs	8	8
Av. initial wt., lb.	34	34
Av. final wt., lb.	195	199
Av. gain, lb.	161	165
Av. daily gain, lb.	1.40	1.44
Av. daily feed, lb.	5.09	5.14
Pounds feed per 100 lb. gain	362.7	356.7

Trial II

Ethomid C-15 failed to exert a growth stimulation when incorporated into a vitamin-fortified, mixed animal and plant protein ration. Animals consuming the basal ration (Lot I) grew at an average rate of 1.51 lb. per day during the 79-day feeding trial. In the positive control group (Lot II) the addition of 7.5 milligrams of aureomycin hydrochloride resulted in a significantly increased ($P<0.01$) average growth rate of 1.71 lb. per day. Pigs receiving 0.025 percent (Lot III),

0.05 percent (Lot IV) and 0.10 percent (Lot V) of Ethomid C-15 in their ration grew at average rates of 1.58 lb., 1.42 lb., and 1.42 lb., respectively, per day.

When alkyl benzene sulfonate was incorporated in the diet at a level of 0.20 percent of the basal ration (Lot VII), pigs consuming this ration grew at a rate (1.68 lb. per day) which was significantly

TABLE 2. TWO SURFACTANTS COMPARED WITH AUREOMYCIN AS A GROWTH STIMULANT FOR SWINE
(Length of Trial—79 Days)

	Treatments	Initial Wt., Lb.	Final Wt., Lb.	Av. Daily Gain, Lb.	Av. Daily Feed, Lb.	Total Feed per Lb. Gain, Lb.
Lot 1	Basal ration	35	154	1.51	4.56	3.0
Lot 2	Basal ration plus 7.5 mg. aureomycin per lb.	35	169	1.71**	5.22	3.1
Lot 3	Basal ration plus 0.025% Ethomid C-15	35	160	1.58	4.51	2.9
Lot 4	Basal ration plus 0.05% Ethomid C-15	35	147	1.42	4.20	3.0
Lot 5	Basal ration plus 0.10% Ethomid C-15	35	147	1.42	4.28	3.0
Lot 6	Basal ration plus 0.10% alkyl benzene sulfonate	35	158	1.56	4.59	2.9
Lot 7	Basal ration plus 0.20% alkyl benzene sulfonate	35	168	1.68*	4.93	2.9
Lot 8	Basal ration plus 0.40% alkyl benzene sulfonate	35	153	1.50	4.38	2.9

* LSMD for daily gain, 5%, 0.15 lb.
** LSMD for daily gain, 1%, 0.20 lb.

greater ($P<0.05$) than that of the pigs on the basal ration. The rate of growth of the pigs consuming 0.20 percent alkyl benzene sulfonate was not significantly different from that of the pigs consuming 7.5 miligrams of aureomycin hydrochloride per pound of total ration. Other levels of alkyl benzene sulfonate (0.10 and 0.40 percent) failed to exert a significant effect on growth rate.

Feed efficiency was not improved by the feeding of aureomycin, alkyl benzene sulfonate or Ethomid C-15.

Discussion

Ethomid C-15 failed to give a growth response when it formed 0.025, 0.05 or 0.10 percent of the diet of weanling pigs in dry lot. Conversely, when alkyl benzene sulfonate was incorporated into the ration of weanling pigs at a level of 0.20 percent, it resulted in a significant growth stimulation over the basal controls and gave results similar to that obtained by incorporating aureomycin hydrochloride at a level of 7.5 mg. per pound of total ration. Differences in growth stimulating properties of the two surfactants reported in this paper concur with the report that detergents vary in their ability to stimulate growth in poultry (Ely and Schott, 1952). In contrast to the work reported in this paper, Ethomid C-15, at a level of 0.1 percent of the ration, caused significantly increased growth rate of swine in trials reported at the Michigan Station (Luecke *et al.*, 1952). These differences in results cannot be fully explained now, but at least it appears that the response of pigs to Ethomid C-15 is not consistent.

The role of surfactants in producing growth stimulation and the relation of the action of surfactants to that of antibiotics is not clear. Ely and Schott (1952) have suggested that the action may be due to selective bacterial inhibition or to the "wetting" action possessed by detergents, or both.

Summary

The inclusion of alkyl benzene sulfonate at a level of 0.20 percent of the ration of weanling pigs resulted in significantly increased growth rate over the basal control group and one which was not significantly different from the response produced by aureomycin hydrochloride (7.5 mg./lb. ration).

At levels of 0.10 or 0.40 percent of the ration, alkyl benzene sulfonate did not affect the growth rate significantly.

Ethomid C-15 at levels of 0.025, 0.05 or 0.10 percent failed to exert a growth stimulating effect.

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COBALT, ALFALFA AND MEAT SCRAPS IN DRYLOT RATIONS FOR GROWING-FATTENING PIGS [1]

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PREVIOUS reports from this station (Klosterman *et al.*, 1950; Dinusson *et al.*, 1951) have demonstrated that additions of cobalt to rations increased gains of growing-fattening pigs in drylot. Robison (1950) also observed that cobalt supplementation to corn-soybean meal rations stimulated gains. Noland *et al.* (1951) report that additions of trace minerals, namely, cobalt, copper, iron, and manganese, increased gains and feed efficiency although the degree of response varied somewhat between experiments. Workers at the Iowa Station (Speer *et al.*, 1952) also noted a small but consistent increase in the rate of gain by additions of trace minerals but no apparent effect on feed efficiency. Both Cornell and Iowa workers suggest that possibly some of the trace minerals contained naturally in feedstuffs used in the rations might not be entirely available for the pig. Limited analysis of the cobalt in feedstuffs indicates forages and hays to contain more of this trace mineral than cereal grains and other concentrates, and previous trials at this station indicate that the cereal grains vary in cobalt content. Therefore, it was deemed advisable to investigate the possible effect of alfalfa and meat scraps, alone and in combination, on the response to cobalt supplementation. The discovery of Rickes *et al.* (1948) that vitamin B_{12} contains cobalt indicates a close relationship between this mineral and vitamin. It is not known whether cobalt functions *per se* or in the formation of B_{12} in the pigs.

Experimental Procedure

One hundred and twenty thrifty Duroc pigs were allotted at random within sex, litter, and weight groups into 20 lots. These pigs had initial weights of approximately 54 pounds and were kept on concrete until they reached market weights of approximately 200 pounds. A factorial design permitting five treatments to be tested on four rations

[1] Published with approval of the Director, North Dakota Agricultural Experiment Station, Fargo; Project Report on Project R&M 10.

was used. These treatments included basal, cobalt, B_{12}, cobalt plus B_{12}, and a commercial trace mineral salt. The composition of the rations is presented in table 1.

All rations were thoroughly mixed in a twin-spiral mixer and self-fed. Weights and fed consumption were taken at two-week intervals. Iodized salt was fed in the first four treatments with cobalt carbonate added, 35 grams per 100 pounds of salt, to the lots where cobalt or cobalt plus B_{12} were the treatments. This inclusion of cobalt as the carbonate added about 885 micrograms of cobalt per pound of ration. The trace mineral salt used as the fifth treatment had a guaranteed percentage analysis of cobalt, 0.01; manganese, 0.25; iron, 0.16;

TABLE 1. RATIONS [1]

	A	B	C	D
Corn (#3 yellow)	72.0	74.0	68.5	71.4
Soybean oil meal (44%)	26.0	20.0	24.5	18.0
Meat scraps (55%)	....	5.0	....	5.0
Alfalfa (21% dehy.)	....	....	5.0	5.0
Salt	0.5	0.5	0.5	0.5
Bonemeal	0.5	....	0.5	....
Limestone	1.0	0.5	1.0	0.1
Percent protein (total)	18.0	18.2	17.9	17.9

[1] Quadrex added to supply 90 I.U. vitamin D and 725 I.U. vitamin A per pound of ration.

copper, 0.033; iodine, 0.007; and zinc, 0.005. Thus, this treatment added about 227 micrograms of cobalt per pound of feed.

It was the intention to add 12 micrograms of B_{12} per pound of ration; after completion of the experiment, however, it was discovered that instead of containing 12.5 milligrams per pound as claimed, the actual content was less than 2 milligrams per pound of commercial vitamin supplement and provided less than 2 micrograms per pound of ration.

The alfalfa used was excellent quality dehydrated meal guaranteed to contain 17% protein but contained 21% by actual analysis. The meat scraps (55% protein) were of good quality, purchased directly from the plant, and still warm from processing, indicating a fresh product. The soybean meal (44% protein) was processed by the trichloro-ethylene method. The corn used was purchased from a local feed concern and was artificially dried to 17–18% moisture, indicating a No. 3 grade.

Results and Discussion

The pertinent results are summarized in table 2. The average daily gains per lot are reported together with the average pounds of feed required per pound of gain, thus allowing for comparisons of differences between rations and treatments.

TABLE 2. AVERAGE DAILY GAIN AND FEED REQUIRED PER POUND OF GAIN

	Rations				
	A	B	C	D	
Treatments	Basal	Basal+ Meat Scraps	Basal+ Alfalfa	Basal+ Meat Scraps+ Alfalfa	Treatment Averages
1. Basal					
Av. daily gain, lb.	1.56	1.61	1.48	1.55	1.55
Av. lb. feed/lb. gain	4.13	4.41	4.47	4.15	4.29
2. Cobalt					
Av. daily gain, lb.	1.65	1.84	1.57	1.72	1.70
Av. lb. feed/lb. gain	4.95	4.37	4.36	4.07	4.44
3. B_{12}					
Av. daily gain, lb.	1.59	1.63	1.53	1.67	1.60
Av. lb. feed/lb. gain	4.28	4.32	4.26	4.55	4.35
4. Cobalt+B_{12}					
Av. daily gain, lb.	1.55	1.72	1.46	1.66	1.60
Av. lb. feed/lb. gain	4.58	4.21	5.09	5.50	4.85
5. Trace mineral salt					
Av. daily gain, lb.	1.65	1.79	1.53	1.62	1.65
Av. lb. feed/lb. gain	4.08	4.58	4.77	4.36	4.45
Rations averages					
Av. daily gain, lb.	1.60	1.72	1.51	1.64	....
Av. lb. feed/lb. gain	4.40	4.38	4.59	4.53	....

As reported previously from this station, the inclusion of cobalt gave a greater response than any other treatment. This increased rate of gain was significant ($P=0.05$) over the basal. The greatest response was obtained when cobalt was added to the rations containing meat scraps. Apparently, there was no interaction, but rather the action of the cobalt and meat scraps was additive. Vitamin B_{12} at the low levels of approximately 2 micrograms per pound of ration had no effect. The cobalt, however, did not promote the same rate of gain when included with this small amount of B_{12} as it did alone. This is consistent on all four rations. However, the differences between

treatments 2 (cobalt alone) and 4 (cobalt plus B_{12}) are not statistically significant and may be due to chance. This point needs further investigation. It is also of interest that treatment 5, a commercial trace mineral salt, did not stimulate gains as much as cobalt alone. The level of cobalt was less in this treatment, adding only 227 micrograms per pound of ration as compared to 885 micrograms for the cobalt alone. It appears that the additional trace minerals provided by the trace mineral salt were not lacking in the basal ration.

Inclusion of 5% meat scraps in rations B and D resulted in highly significant (P=0.01) increases in rate of gain for the pigs in the lots so fed over the "all plant" type rations. It is felt that this was due to a more complete balance in amino acids rather than addition

TABLE 3. STATISTICAL ANALYSIS OF AVERAGE DAILY GAINS FROM 55 TO 200 POUNDS

Sources of Variation	Degrees of Freedom	Mean Square (Coded)	F Value
Total	112	.....	.....
Rations	3	1782.68	8.88**
Treatments	4	596.62	2.97*
Av. response to alfalfa	1	1598.70	7.97**[1]
Av. response to meat scraps	1	3740.83	18.64**
Meat scraps × alfalfa	1	8.53	.....
Treatments × rations	12	100.25	.....
Error	93	213.66	.....

* Significant at p=0.05.
** Significant at p=0.01.
[1] Negative response.

of additional B_{12} or any other vitamin which may have been contained therein. It was thought that the alfalfa might improve the ration by providing additional tryptophane to the ration which possibly was on the borderline in this amino acid, but this was not the case. Meat scraps gave the greatest increase in gains where cobalt was added. On the other hand, additions of excellent quality dehydrated alfalfa (analysis 21% protein) significantly (P=0.01) decreased the rate of gain. A similar trend has been noted in other experiments (unpublished data). Apparently, alfalfa reduces the palatability and promotes "sorting" and waste of feed. However, the deleterious effect of the alfalfa is greater than can be explained on this basis. The depressing effect of alfalfa on the growth of chicks has been reported by Draper (1948). Much of the work on this subject has been reviewed (Nutrition Review, 1951). It is entirely possible that inclusion of alfalfa

in the rations of growing-fattening pigs might have a similar action.

Feed efficiency was noticeably poor in all lots. Inasmuch as under farm conditions any feed rooted out of feeders is waste and yet must be charged against the pigs, no attempt was made to weigh back the sorted and wasted feed. The feed requirements were noticeably, though not statistically, greater for those lots receiving alfalfa in the ration.

The data were subjected to statistical analysis and the pertinent summary is reported in table 3. Seven pigs were removed from the experiment because of prolapse of the rectum. These occurrences were at random and showed no correlation to rations or treatments. This accounts for the reduced degrees of freedom as shown in the table. The error term as shown is the remainder after all sources of variation were removed.

Summary

1. Cobalt supplementation increased significantly the rate of gain in growing-fattening swine.

2. Addition of 5% meat scraps to a corn-soybean meal type ration increased significantly the rate of gain; the greatest increase was noted in the pigs which also received cobalt.

3. Inclusion of excellent quality dehydrated alfalfa as 5% of the ration depressed significantly the rate of gain of growing-fattening pigs.

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A CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF COBALT IN FEEDS [1]

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COBALT is one of the essential minor elements in the nutrition of ruminants (Filmore and Underwood, 1937). Recently cobalt has been shown to stimulate gains in growing-fattening swine (Dinusson *et al.*, 1951), and has been found to be a constituent of vitamin B_{12} (Rickes *et al.*, 1948). Pastures and forages deficient in cobalt for the ruminant have been reported in many areas, while cereal grains appear to contain even less cobalt than some roughages. The role of cobalt in animal nutrition should be given greater emphasis for efficient livestock production.

A large number of methods and modifications of previous methods for cobalt analysis have been published. However, there is a decided need for improvement in the determination of cobalt in feeds.

One distinct disadvantage of all previous methods for the cobalt determination in feeds is that the small amounts of cobalt present make it almost impossible to obtain enough ash in solution for a reliable colorimetric determination. This disadvantage is overcome by passing a perchloric acid solution of the plant ash containing a cobalt nitroso R salt complex through a perchloric acid-washed alumina adsorption column and concentrating the cobalt complex on the column.

Dean (1951) proposed the use of a perchloric acid-washed alumina column for the determination of cobalt in ores. Without certain modifications in this method it was found that the cobalt nitroso R salt complex would pass through the column because of interfering substances, such as excess sulfates, phosphates, and pyrophosphates. The sulfates are removed by precipitation with barium chloride, the pyrophosphates are hydrolized to orthophosphates by perchloric acid, and the eluting effect of the orthophosphates is inhibited by increased perchloric acid concentration of the cobalt nitroso R salt complex solution.

[1] Published with permission of the Director of the North Dakota Agricultural Experiment Station.

[2] This paper is part of a thesis to be submitted to the Graduate Council of the North Dakota Agricultural College in partial fulfillment of the requirements for the degree of Master of Science.

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The method described below employs the use of a perchloric acid-washed alumina adsorption column and the nitroso R salt as the specific reagent for cobalt. The necessity for the removal of iron and copper is eliminated. This method has been successfully applied to a number of feeds.

Materials and Method

Apparatus

An Evelyn photoelectric colorimeter with a 520 millimicron filter.

A Beckman pH meter, model G, or pH test papers with a pH range from 3.0 to 5.5.

A Martin M-15500 chromatographic tube—D 19 mm., L 200 mm.

Reagents

A standard solution of cobalt (1 mg. per ml.) is prepared by dissolving 0.263 grams of cobalt sulphate in distilled water and adding 10 ml. of 72% perchloric acid, and then diluting to 1 liter. This solution is then diluted so that it contains 10 micrograms of cobalt per milliliter.

A 4 N sodium acetate solution is prepared by dissolving 328 grams of C. P. salt in distilled water and diluting to 1 liter.

A 1 percent nitroso R salt solution is prepared by dissolving 10 grams of the nitroso R salt in 1 liter of distilled water.

Fischer adsorption alumina (80–200 mm.) is used. The material is repeatedly washed with water and the finely divided portions decanted and discarded until only a rapidly settling product is obtained. This product is air dried and stored in a glass bottle.

Preparation of Column

A tuft of glass wool is packed at the bottom of the tube. A water slurry containing 5 grams of the washed adsorption alumina is added and packed in the tube. Another tuft of glass wool is packed on top of the solid alumina column. A glass stopcock is attached to the lower end of the tube and a burette tip then attached to the stopcock. A 500 ml. separatory funnel is attached to the upper end of the adsorption tube by means of a rubber stopper connection. The flow of liquid through the column may then be controlled by either of the two stopcocks.

The alumina column is treated with 1 M perchloric acid until the

effluent is distinctly acid. It is then ready to be used. The column is recharged by treating it with 2 M sodium hydroxide until the effluent is distinctly alkaline, washing with distilled water, and then treating again with 1 M perchloric acid until the effluent is distinctly acid.

Procedure

From a uniform sample ignite 5–100 grams in a porcelain crucible overnight in a muffle furnace at temperatures not exceeding dull redness. If ashing is not complete, moisten the ash with 8 N nitric acid. Evaporate to dryness and ash again in the muffle furnace. Repeat the nitric acid treatment if necessary. Cover the crucible with a watch glass and then add 10–20 ml. of 6 N hydrochloric acid. Heat on a hot plate, then remove the watch glass and evaporate the contents to dryness. Dissolve the acid-soluble ash with 6 N hydrochloric acid with the aid of heat on a hot plate, and then transfer the contents quantitatively to a beaker. Boil the contents in the beaker, and filter through a Whatman No. 31 filter paper to remove the insoluble silicates and small amounts of unoxidized carbon present. To the filtrate add a sufficient quantity of a 10% barium chloride solution and set on a hot plate to allow the precipitated sulfates to crystallize and settle. Filter to remove the barium sulfate. Collect the filtrate in a 400 ml. beaker and evaporate to dryness, or make the filtrate up to volume, take an aliquot and evaporate to dryness.

Add 5–15 ml. of 72% perchloric acid to the dry residue. The amount of perchloric acid added depends upon the amount of ash present. Allow the contents to fume vigorously for 3 to 5 minutes. Do not fume to dryness. Fuming to dryness may cause the formation of pyrophosphates. Cool slightly and then add approximately 50–100 ml. distilled water. Heat to get the salts into solution. Add 5 ml. of 3% hydrogen peroxide. Boil the contents a few minutes to convert any pyrophosphate present to the orthophosphate and remove the excess hydrogen peroxide. Cool to 50–60° C. Add 10–15 ml. of the 1% nitroso R salt solution. Adjust the pH of the solution to between 5.0 and 5.5 with the addition of 4 N sodium acetate. Heat the solution slowly to boiling with constant stirring. Remove from the burner. Add 0.5 ml. of 72% perchloric acid for each ml. of 4 N sodium acetate used, plus an additional 20 ml. of the 72% perchloric acid. Dilute the solution to approximately 400 ml. with distilled water and

cool to room temperature. Transfer the solution to the separatory funnel which is attached to the adsorption column. Wash out the beaker with a few ml. of 1 M perchloric acid. (At this particular stage for some feeds, e.g., linseed meal, there is often a heavy potassium perchlorate precipitate. This precipitate does not affect the cobalt determination, but if poured directly into the separatory funnel it will settle to the bottom and clog the adsorption column. This difficulty is overcome by allowing the precipitate to settle to the bottom of the beaker, and decanting the clear supernatant liquid into the separatory funnel. After this liquid has passed through the column, the precipitate is washed directly into the column with 1 M perchloric acid. This precipitate is readily soluble in the dilute nitric acid used later to elute the excess nitroso R salt.)

Adjust the flow through the column at the rate of 7–12 ml. per minute. After the solution has passed through the column, wash the separatory funnel and column with a few ml. of 1 M perchloric acid. Wash the column with 0.5 N nitric acid until most of the excess nitroso R salt is removed. Then wash the column with 1 N nitric acid solution heated to 80° C. until the eluate is colorless. Elute the cobalt nitroso R salt complex from the column with 2 M sulfuric acid into a 25 or 50 ml. volumetric flask. Allow the acid to pass slowly through the column at the rate of 3 ml. or less per minute until the cobalt has been eluted from the adsorption alumina. Then increase the rate of flow of the acid. If the acid is allowed to run slowly through the column, the cobalt can be eluted with 25 ml. or less of acid. Make up to volume. If the solution is cloudy, centrifuge or filter through a dry filter paper (Whatman No. 42) into a colorimetric tube. Determine the transmittancy of the 2 M sulfuric acid cobalt solution at a wave length of 500–520 millimicrons. The cobalt complex is stable for 48 hours in the absence of excess dye (Dean, 1951). Calculate the quantity of cobalt present from a standard calibration curve which has been prepared from known amounts of cobalt and reagents similarily developed and chromatographed.

Results

The cobalt content of feeds (p.p.m.) analyzed by the chromatographic method are given in table 1. The size of the sample used and the variation in the cobalt for duplicate samples are also given in table 1. Recovery experiments were made by the addition of 10 micrograms

of cobalt to the perchloric acid solution of the plant ash. The percent recovered averaged 98±3%.

Orthophosphates, if present in too great a quantity, interfere with

TABLE 1. COBALT CONTENT OF FEEDS

(Moisture Free Basis)

Sample	Sample No.	Sample Wt.	Average Cobalt Content	
			Found	Reported
		gm.	p.p.m.	p.p.m.
Beet pulp	1	25	0.322	0.14–1.02[1]
Barley, grain	2	50	0.027	0.05–0.05[1]
Barley, grain	3	100	0.091	0.05[1]
Barley, ground	4	100	0.044	0.21–0.24[1]
Bran (wheat)	5	100	0.043	0.01[1]
Corn (yellow #2)	6	100	0.019	0.01–0.02[2]
Corn (yellow #2)	7	100	0.014	.....
Corn (yellow #2)	8	100	0.027	.....
Cottonseed meal (37%)	9	50	0.329	.....
Cottonseed meal (37%)	10	50	0.238	.....
Fish meal	11	25	0.118	.....
Linseed meal (37% exp.)	12	50	0.566	.....
Meat scraps (55%)	13	25	0.135	.....
Meat scraps (55%)	14	25	0.160	.....
Meat scraps (55%)	15	25	0.097	.....
Middlings (wheat)	16	50	0.116	0.14–0.16[1]
Oats, grain	17	50	0.040	0.05–0.07[1]
Peas, Alaska	18	50	0.114	.....
Soybean meal				
Cooked (solvent)	19	50	0.190	.....
Cooked (solvent)	20	50	0.272	.....
Raw	21	50	0.221	.....
Sunflower meal	22	50	0.500	0.13–0.14[1]
Wheat, grain	23	100	0.077	0.01[3]
Alfalfa hay (good)	24	50	0.266	0.01[4]
Alfalfa, dehydrated (17%)	25	25	0.468	0.38–0.40[1]
Barley hay (early cut)	26	25	0.062	.....
Brome grass	27	25	0.108	.....
Oat hay (early cut)	28	25	0.047	0.02[4]
Oat hay (late cut)	29	25	0.085	.....
Prairie hay (upland)	30	25	0.130	.....
Prairie hay (upland)	31	25	0.128	.....
Prairie hay (upland)	32	25	0.120	.....
Prairie hay (upland)	33	25	0.097	.....
Crested wheat grass	34	25	0.224	.....
Crested wheat grass	35	25	0.250	.....

[1] Campen and Queerling, 1952.
[2] Hurwitz and Beeson, 1944.
[3] Bertrand and Mokragnatz, 1930.
[4] Bowstead, Sackville, and Sinclair, 1942.

the adsorption of the cobalt complex on the adsorption column. The interference of the orthophosphate but not the pyrophosphate can be inhibited by adding an excess of perchloric acid, providing the quantity of orthophosphate present is less than the equivalent of 1 gram of the elemental phosphorus. In a feed sample containing 4 percent phosphorus, e.g., meat meal, no more than 25 grams per sample should be used for analysis under the conditions described in the procedure.

If severe bleeding occurs or the cobalt complex is not adsorbed at the top of the adsorption alumina column, it indicates the presence of excess orthophosphates, or that pyrophosphates are present. All pyrophosphates present must be converted to the orthophosphate, or better, eliminate as much as possible the conditions that are favorable to the formation of pyrophosphates.

Any organic matter or partially oxidized organic matter must be absent from the solution previous to the addition of the nitroso R salt. The presence of any organic matter will give a high fictitious cobalt result. All iron must be oxidized to the ferric state.

The nitroso R salt is added in excess to insure that there is enough of the complexing reagent to combine with the cobalt in case other competing substances are present, e.g., copper and iron. The presence of the excess nitroso R salt does not interfere with the colorimetric determination as it is easily eluted from the adsorption alumina prior to the elution of the cobalt complex.

The advantage of this method over other methods is that larger samples of feed may be taken for analysis, and the method is also more specific for cobalt. The use of a perchloric acid-washed alumina adsorption column makes the nitroso R salt a specific reagent for cobalt. The necessity for the removal of iron and copper is eliminated.

The cobalt determinations reported in table 1 are those of a random selection of feedstuffs. The repeatability of results on feed samples from the same source is within normal experimental error. However, it was noted that a greater variation between determinations of similar feedstuffs from different sources occurred which suggested that feeds, in general, may vary greatly in cobalt content. These data indicate that cereal grains and animal proteins are a poor source of cobalt. Protein concentrates of plant origin appear to contain greater amounts of cobalt than the cereal grains. Roughages, particularly legumes and beet pulp, contain larger amounts of cobalt than the common grasses.

Summary

A chromatographic method for the determination of cobalt in feeds is described. The ashed sample is dissolved with HCl, filtered, and the filtrate evaporated to dryness. Perchloric acid is added and the contents evaporated to white fumes. With the addition of distilled water and nitroso R salt, the cobalt complex color is developed in the usual manner. The entire solution is passed through a perchloric acid-washed alumina column. The cobalt complex is adsorbed on the column. The excess reagent is eluted from the column with dilute HNO_3. The cobalt complex is then eluted with dilute H_2SO_4, and determined colorimetrically.

Advantages over previous methods are that copper and iron do not interfere with the cobalt determination, and larger initial samples may be ashed to give a greater concentration of cobalt in the final solution for a colorimetric determination.

As determined by this method, the cobalt content of hays range from 0.047 to 0.468 p.p.m., cereal grains from 0.014 to 0.091 p.p.m., and plant protein concentrates (sunflower, cottonseed, and linseed meal) from 0.190 to 0.500 p.p.m.

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THE APPARENT DIGESTIBILITY OF NITROGEN AS ASSOCIATED WITH CALCIUM FERTILIZATION OF THE SOIL [1]

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THE relation of the soil to the composition of plants has long been a subject of interest. The vast volume of evidence relating to this general subject has been ably summarized (Beeson, 1941). The chemical composition of forages in Illinois has been shown (Snider, 1946) to vary with soil fertilization. Relatively few studies have been reported which demonstrate a relation between soil fertilization and the degree to which animals may utilize nutrients as produced under differing soil fertilizing systems.

Animal feeding studies have shown increases in the percentage of digestible protein in pasture forage (Pipers, 1909), as well as higher vitamin B content in wheat (Hunt, 1927), to be associated with soil fertilization. In other animal feeding studies (Crampton, 1934; Blaser *et al.*, 1943; Webb *et al.*, 1946) increased gains in body weight have been shown to be associated with higher levels of soil fertility. Other studies (Nehring, 1938; Eheart and Pratt, 1942) have revealed an increase in the apparent digestibility of pasture herbage which was related to higher soil fertility levels attained by fertilizer application.

A greater lactation response has been demonstrated where calcium was applied to a phosphorus-potassium fertilized soil (Kendall *et al.*, 1948). Unpublished evidence gained in that study indicated that a significantly higher coefficient of apparent digestibility of nitrogen was associated with a high rate of calcium application to the soil. This may have been a factor contributing to the results of that study.

The purpose of this report is to present the accumulated evidence of an increased apparent digestibility of nitrogen in lespedeza hay and wheat as being associated with calcium fertilization.

[1] An abstract of this paper was presented at the 47th Annual Meeting of the American Dairy Science Association, June 1952, at Davis, California.

[2] Deceased.

[3] The authors are most grateful to Professor P. E. Johnson of the Agronomy Department, University of Illinois, for his careful supervision of the harvest of the wheat and lespedeza crops used in the preparation of rations and to E. E. Hixon and G. D. Rolleri for their technical assistance in the conduct of the study.

[4] Department of Dairy Science.

Experimental

The coefficients of apparent digestibility of dry matter and nitrogen in Korean lespedeza hay and wheat grain produced under two systems of fertilizer treatment were determined. The crops were grown on the Newton (Illinois) Soil Experiment Field which is supervised by the Department of Agronomy, University of Illinois. The soil of the field consists of a single soil type, Cisne silt loam. Accurate records of fertilizer application have been kept since the establishment of the field in 1912. One system of fertilization (CaPK) included a total of 12 tons of limestone, 4 tons of raw rock phosphate, 2.25 tons of kainit, and 1000 pounds of muriate of potash per acre, while the other system (PK) was similar except that no limestone had been applied.

Two digestion studies, using the rabbit as the experimental animal, were conducted. Chemical composition of all diets and feces were determined by standard procedures (AOAC, 1945).

Experiment 1

The lespedeza produced under the CaPK and PK systems of soil fertilization was cut in the early bloom stage and cured with a minimum of leaf loss. The hay was baled and stored in a mow until used. All foreign matter was removed from the lots of lespedeza hay before grinding. Each of the two lots of hay and each of the two lots of wheat produced under the same systems of soil treatment were separately ground in a hammer mill. Fine grinding of these feeds prevented the animals from sorting the ingredients in the diets.

The diets consisted of 49.5% lespedeza hay, 49.5% wheat and 1% sodium chloride. The diets were thoroughly mixed and were identified as CaPK and PK, respectively, according to the soil treatment system under which the feeds were grown.

Ten New Zealand White female rabbits of similar size were paired, using litter mates to form a pair, and were pair-fed with equalized food intakes (Mitchell and Beadles, 1930). Water was available at all times. After the diets had been fed for a period of 33 days, a 5-day collection period was conducted, during which ferric oxide was used as a fecal marker. The feces were collected daily and were dried at 60° C. After drying, they were ground and kept under air-dry conditions until analyzed.

Experiment 2

The lespedeza hay and wheat used were produced on the same field as was that in experiment 1 and under the same CaPK and

PK soil treatments. The lespedeza was cut at "early" hay and "late" hay stages of development, 3 weeks having elapsed between cuttings. The lespedeza was harvested from adjacent plots, and when only slightly wilted was placed in a ventilated mow where it was cured without leaf loss due to handling. The hay was virtually weed-free, and the wheat was harvested and stored under ideal conditions. After the hay had been thoroughly cured, the four lots of hay and the two lots of wheat were finely ground in a hammer mill. The hays and the diets in which they were used were identified as CaPK early, PK early, CaPK late, and PK late.

In part 1 of experiment 2, the four lots of finely ground lespedeza hay were fed to four pairs of New Zealand White rabbits in a trial having a 4 x 4 Latin square design. *Ad libitum* feeding of the hay to which 1% sodium chloride (C.P.) had been added extended over a 2-week period with daily measurement of the feed consumed. Fecal collections were made daily. Analyses for dry matter and total nitrogen were made on samples of feces composited for each rabbit on a weekly basis. Coefficients of apparent digestibility of dry matter and total nitrogen were calculated on the basis of weekly diet intake and weekly fecal output.

Unfortunately, five of the eight animals either refused to eat the hay or consumed so little of it that measurable results were obtained on only three animals.

In part 2 of experiment 2, the lespedeza hay was mixed with wheat grown under the same soil treatment. Four pairs of litter-mate rabbits of the Dutch breed (approximately 4 months of age) were used as experimental animals. The experimental design was a 4 x 4 Latin square in which each of the four pairs of animals received each of the four rations. There were four feeding periods of 21 days each, in which a 7-day preliminary feeding period was followed by a 14-day fecal collection period. Each animal was offered daily a weighed quantity of diet which was in excess of the amount it would consume during 1 day, thus permitting a study of diet intake. The amount of diet remaining in each feeder as well as any portion that may have been spilled was weighed daily. The type of feeder used was so constructed that it reduced to a minimum the spilling and scratching out of the diet. The animals were fed daily, immediately after the fecal collections were made, and water was available at all times.

Fecal collections were made at approximately 5 p.m. each day. Each animal's daily fecal output was placed in an individual, cello-

phane bag. The collection bags were tightly rolled to prevent moisture loss and were frozen and stored at —20° C. until analyzed for dry matter and total nitrogen. In preparing the frozen feces for analysis, each daily collection was pulverized and thoroughly mixed. Aliquots of the fresh material, approximately 2.5 grams, were used in determining total nitrogen and dry matter. The 14 daily fecal collections made for each diet fed during the four periods were analyzed individually.

TABLE 1. COMPOSITION OF LESPEDEZA AND WHEAT DIETS AS FED

Experiment and Ration	Dry Matter	Protein [1]	Ether Extract	Crude Fiber	N.F.E.	Ash
	%	%	%	%	%	%
I						
CaPK	89.9	13.50	1.63	16.1	54.3	4.40
PK	89.3	10.88	1.53	15.3	56.9	4.66
II						
CaPK early	87.5	11.50	2.08	16.9	53.2	3.86
PK early	87.9	11.25	2.09	15.6	54.6	4.34
CaPK late	87.9	10.56	1.90	17.8	53.9	3.71
PK late	88.0	10.44	2.18	16.9	54.5	3.97

[1] Total nitrogen × 6.25.

Results and Discussion

Experiment 1

The composition of the diets is presented in table 1. The coefficients of apparent digestibility of nitrogen and dry matter in these diets are given in table 2. The mean coefficients of apparent digestibility of nitrogen in the CaPK diet was 55.6% as compared with a coefficient of 40.6% for the PK diet (equal quantities of each diet were consumed). The average total consumption of diet per animal for the 5-day period was 705 grams.

As based on daily refusals of the diets, there seemed to be some tendency for the animals fed the PK diet to refuse a greater quantity of the diet, thus limiting the intakes of their pair mates receiving the CaPK diet. The presence of weeds in the PK hay prior to their removal may have been a factor in influencing acceptability by imparting an objectionable odor to the hay.

A *t* test of the coefficient of apparent digestibility observed in this trial revealed a highly significant difference in apparent digestibility

of nitrogen in favor of the CaPK system of fertilization. There was no significant difference between diets in the amount of dry matter digested.

Experiment 2

Part 1. No significant differences in the apparent digestibility of the dry matter were observed between the CaPK early and the PK early hays. The coefficients of apparent digestibility of nitrogen for these two lots of hay were 50.3% and 47.1%, respectively, and were

TABLE 2. AVERAGE ANIMAL RESPONSE VALUES AS RELATED TO SOIL TREATMENT AND STAGE OF LESPEDEZA DEVELOPMENT AT HARVEST

Soil Treatment and Ration	No. of Animals	Dry Matter			Nitrogen			14-Day Weight Gains
		Daily Intake	Digested	Coeff. Dig.	Daily Intake	Digested	Coeff. Dig.	
		gm.	gm.	%	gm.	gm.	%	gm.
Experiment 1: Hay and Wheat								
CaPK	5	141.2	92.4	68.0	3.05	1.68	55.6	....
PK	5	140.8	92.5	68.2	2.45	0.99	40.6	....
Experiment 2								
Part 1: Hay Only								
CaPK early	1	108.3	62.9	58.1[1]	2.56	1.29	50.3[1]	....
PK early	1	78.2	44.9	57.4[1]	1.91	0.90	47.1[1]	....
CaPK late	1	90.1	49.4	54.8[1]	1.83	0.82	45.2[1]	....
Part 2: Hay and Wheat								
CaPK early	8	81.3	47.3	58.5	1.69	0.72	42.6	50.2
PK early	8	81.9	47.5	58.1	1.66	0.62	37.2	61.5
CaPK late	8	82.2	48.4	59.2	1.55	0.57	36.6	33.5
PK late	8	75.3	42.9	47.2	1.44	0.47	32.5	20.4

[1] Calculated on the basis of weekly diet dry matter intakes and weekly amount of dry matter or nitrogen removed as determined from the weekly composited fecal collections.

significantly different at the 5% level of probability. However, the differences are confounded with rabbits.

Part 2. As related to soil treatment and stage of development, the ratio of wheat nitrogen to lespedeza nitrogen in the CaPK early, PK early, CaPK late and PK late diets were 1:1.265, 1:1.446, 1:1.086, and 1:1.309, respectively. The average animal response with reference to the amounts of dry matter and total nitrogen digested appear in table 2. The amount of dry matter digested in the case of the diets that were produced under the CaPK system of soil treatment was slightly greater than that for the diets produced under the PK system.

An analysis of variance revealed this difference to be significant at the 5% level of probability. The apparent digestibility of the nitrogen showed a highly significant difference in favor of the diets produced on the CaPK system. The analyses of variance of these data are summarized in table 3 (Snedecor, 1946). No significant differences were found in the tendency of the animals to prefer one ration over any other as shown by their levels of food intake.

The average gain in body weight per animal appears in table 2. The mean body weight of the animals showed a slight increase throughout the test periods, indicating that the animals were in positive energy balance.

TABLE 3. THE ANALYSES OF VARIANCE FOR DRY MATTER INTAKE, DRY MATTER DIGESTED, PERCENT NITROGEN DIGESTED, AND WEIGHT GAINS (PART 2 OF EXPERIMENT 2)

Source of Variation	Degrees of Freedom	Mean Squares			
		Feed Intake	Percent Dry Matter Digested	Percent Nitrogen Digested	Weight Gain
Pairs	3	4505*	281.7**	376.0*	0.77
Periods	3	5302*	27.8	1317.2**	0.85
Rations	3	1045	78.1	1922.6**	0.26
CaPK vs. PK	1	678	167.7*	2486.8**	0.10
Early cut vs. late cut	1	1272	0.5	3237.3**	0.67
Interaction	1	1186	66.2	43.7	0.12
Error	6	905	20.3	58.8	0.29

* Significant at the 5% level of probability.
** Significant at the 1% level of probability.

It appears that under the conditions of these studies the coefficients of apparent digestibility of nitrogen in diets prepared from feeds that had been grown on a calcium-phosphorus-potassium fertilized soil were significantly greater than those of diets composed of feeds that were produced on a phosphorus-potassium treated soil. The "early" hay stage of development of the forage when harvested likewise had a highly significant effect upon the apparent digestibility of nitrogen. No significant differences in dry matter intake could be attributed to the calcium treatment of the soil.

Studies are under way in an effort to throw further light on this problem which is believed to have fundamental importance.

Summary

Experiments are described in which the apparent digestibility of the nitrogen in lespedeza hay and in wheat grain was significantly higher when these crops were grown under a calcium-phosphorus-potassium fertilizer system than when the crops were grown on adjoining plots given a phosphorus-potassium-fertilizer treatment. The rabbit was used as the experimental animal.

The stage of development of the lespedeza at the time of harvest for hay had a highly significant effect upon nitrogen digestibility, a higher coefficient of apparent digestibility being shown for early harvest than for late harvest.

No significant differences were observed between the dry matter intakes for the CaPK and the PK diets.

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NEWS AND NOTES

The forty-fifth annual meeting of the American Society of Animal Production will be held in the Hotel Sherman in Chicago, Illinois on November 27 and 28 with a dinner for the honored guest on November 29.

A. E. Darlow, formerly head of the Animal Husbandry Department at Oklahoma A. & M. College was appointed Dean of Agriculture, Director of the Experiment Station and Vice President in charge of Agricultural Sciences at the institution on February 1. As Vice President he will be in charge of the School of Agriculture, the Agricultural Experiment Station, the Agricultural Extension Service and the School of Veterinary Medicine.

At USDA's seventh annual award ceremonies held in Washington, May 19, T. C. Byerly, head of the Animal Husbandry Division, BAI, received a superior service award.

J. W. Pou, who has been head of the Dairy Husbandry Department at the University of Maryland, was appointed head of the Animal Industry Department at North Carolina State College on July 1. He succeeds D. W. Colvard, who became Dean of Agriculture on that date.

John L. West, formerly at the University of Tennessee, is now Associate Professor of Animal Pathology in the Department of Animal Husbandry at the Pennsylvania State College.

Julius E. Nordby, Director of the U. S. Sheep Experiment Station and Western Sheep Breeding Laboratory, Dubois, Idaho, is on a two year leave of absence to head TCA's program for livestock improvement in Egypt. Clair E. Terrill will be acting Director of the Dubois station.

The retirement of Franklin L. Bentley on June 30 as head of the Department of Animal Husbandry at the Pennsylvania State College, and the appointment of William L. Henning to succeed him, have been announced by Milton S. Eisenhower, president of the College.

"Zebu Cattle of India and Pakistan" by N. R. Joshi and Ralph W. Phillips was published by the Food and Agriculture Organization of the United Nations in March, 1953. It is intended to supply information on the adaptability of various types of Zebu cattle to the environmental conditions of tropical and sub-tropical areas. It contains a brief introduction to the problems of breeding for adaptability to the environment, and a classification of the Zebu cattle of India and Pakistan into six major groups. This is followed by a detailed description of each of the 28 important breeds.

"The Organization of Agricultural Research in Europe" has been issued by the Food and Agriculture Organization of the United Nations, as FAO Development Paper No. 29. It was edited by Ralph W. Phillips, I. Moskovits and F. F. Lininger. It contains material of general interest to animal husbandry workers on the patterns and problems of research organization and financing, and of more specific interest regarding the institutions responsible for livestock research in most European countries.

The Western Section of the American Society of Animal Production had very successful meetings July 27, 28, and 29, at Colorado A. & M. College. A total of 46 scientific papers was presented. The meetings were held jointly with the Western Division of the American Dairy Science Association. The 1954 meetings will be held at Corvallis, Oregon, with Oregon State College as the host institution. New officers are H. Heitman (California), president; L. E. Washburn (Colorado), vice-president; and F. F. McKenzie (Oregon), secretary-treasurer.

P. G. Hogg, Assistant Superintendent of the Delta Branch of the Mississippi Experiment Station has been sent by FAO to British Guiana for a 6 months' period.

R. A. Alexander, Director of Veterinary Services, Union of South Africa, arrived in the United States April 20 to advise BAI and experiment station specialists with respect to the sheep disease, blue tongue. He made field surveys in California, Utah and Texas.

The Rockefeller Foundation has made a $170,000 grant to support research in quantitative genetics carried on by the Department of Statistics, Agricultural Experiment Station, North Carolina State College.

General Physiology by Bradley T. Scheer was published by John Wiley & Sons, Inc., New York in April, 1953. This textbook presupposes a working knowledge of general biology, general and organic chemistry, and the principles of physics. Its five sections have to do with the physicochemical foundations of life, the chemical dynamics of life, energy transformation in cells and organisms, the development of organisms and the integration of the organism. The book is intended to bring before the student new ideas, to introduce him to a field of knowledge, and to encourage him to pursue it farther.

R. M. Jordan, Associate Professor of Animal Husbandry at South Dakota State College, received the degree of Doctor of Philosophy in Animal Nutrition at Kansas State College. At the present time he is in charge of sheep investigation work at South Dakota State College.

Essentials of Physiological Chemistry by A. K. Anderson was published by John Wiley and Sons, Inc., New York, in May, 1953. It is the fourth revised edition of this text book and among the changes is the inclusion of a section on isotopes, a marked revision of the chapters on enzymes, carbohydrate metabolism, chemotherapy and adrenal hormones, and emphasis being given to the physiological action of vitamins.

The Connecticut Agricultural Experiment Station has announced appointment, under a National Science Foundation grant, of David G. Wilson to its Biochemistry Department staff. He received his Ph.D. from the University of Wisconsin and his M.S. degree from Queens University, Kingston, Ontario, Canada.

James W. Wilson, Director Emeritus of the South Dakota Agricultural Experiment Station, often spoken of as "The Father of Animal Husbandry at South Dakota State College," died February 26, at the age of 82.

E. B. Hart, world-renowned biochemist of the Wisconsin Experiment Station, died suddenly at his home in Madison on the morning of March 12, aged 78.

H. S. Willard, Dairy Husbandman of the Wyoming Experiment Station, was granted a year's leave of absence, commencing June 1, to go on a TCA assignment with headquarters at Tel-Aviv, Israel.

S. O. Fladness, BAI's Assistant Chief for Disease Control and Eradication, died in Washington on May 5.

H. H. Sommer, Professor of Dairy Industries at the University of Wisconsin since 1920 and noted for outstanding research in dairy manufacturing, died suddenly at Madison on May 8.

C. A. Iverson, Head of the Dairy Industry Department at Iowa State College, was awarded an honorary doctor of science degree by the South Dakota State College.

George E. Stoddard (B.S. Univ. of Idaho, 1943; M.S. and Ph.D. Univ. of Wisconsin 1948 and 1950 respectively), formerly Assistant Professor of Animal Husbandry at Iowa State College, is now Associate Professor of Dairy Husbandry at Utah Experiment Station.

W. T. Magee, who recently received his Doctorate in genetics and animal breeding, is now Assistant Animal Husbandman at the Sonora station, a branch of the Texas Agricultural Experiment Station.

A publication entitled "Report of the Second Inter-American Meeting on Livestock Production" has been issued by the Food and Agriculture Organization of the United Nations as Development Paper No. 33. It is based on a Meeting held at Bauru, Brazil, from 8–15 December, 1952, in which representatives of 19 countries participated.

Principles of Farm Management by J. N. Efferson was published by McGraw-Hill Book Co., New York in April, 1953. Principles having a common application to all areas and systems of farming are presented to form the basis for a first course for students in Agriculture.

Social Behavior in Animals by N. Tinbergen was published by John Wiley & Sons, Inc., New York in June 1953. Its aim is to present a biological approach to the phenomena of social behavior.

Robert W. Colby, animal nutritionist of the Agricultural Chemical Research Laboratory at The Dow Chemical Company's Midland, Michigan plant, has been appointed director of Agricultural Research for Dow's Texas division.

F. G. Helyar, Professor of Animal Husbandry at Rutgers University, retired May 13, 1953.

Lewis T. Smith is now Assistant in the Animal Industry Department at North Carolina State College.

O. D. Butler, Jr., has returned to Texas A. & M. College in charge of meat investigations in the Animal Husbandry Department, following completion of work for a Doctorate degree at Michigan State College.

A. Mark McKinney, Instructor and Assistant Animal Husbandman at the University of Arizona, has resigned to engage in farming.

J. E. Ingalls, Assistant Animal Husbandman at the San Juan Basin Branch Station, Colorado Agricultural Experiment Station, resigned February 15, 1953.

Aldar F. Kish, Instructor in the Department of Animal and Poultry Industry at the University of Delaware, resigned March 31, 1953 to accept a position with the Farm Bureau Cooperative in Pennsylvania.

Katherine M. Boney, Assistant Chemist in the Department of Animal Husbandry and Nutrition at the University of Florida, resigned January 9, 1953.

Malcolm D. Lane has been appointed Assistant in the Department of Animal Science at the University of Illinois.

Sue Whitford has been appointed Junior Assistant in the Animal Husbandry Department at Purdue University.

Beryl T. McClain, Assistant in Animal Husbandry at the University of Kentucky, resigned January 10, 1953. He is now employed at Coldstream Farm, Lexington, Kentucky.

Walther E. Thomas, Assistant Professor and Assistant Animal Husbandman at the University of Kentucky, resigned February 28, 1953, to manage a farm in Maryland.

Charles J. Heidenreich, Assistant, and R. B. Sleeth, Instructor, are new members of the Animal Husbandry Department, University of Missouri.

J. E. Nutcher, formerly Instructor in the Department of Animal Industry and Range Management, Montana State College, resigned July 31, 1953.

INFORMATION FOR CONTRIBUTORS

1. Papers may be submitted reporting work whose ultimate objective is clearly the solution of a livestock production problem and whose contents should be of interest particularly to those dealing with livestock production problems. Review papers on subjects of general interest will also be published at intervals, and will be prepared on invitation by the Editorial Board.

2. All papers will be subject to critical review by the Editorial Board, or others designated by the Editor. Papers needing revision will be returned to authors and should be revised and returned promptly. Papers not suitable for publication will be returned to authors with a statement of reasons for not accepting them.

3. Papers submitted should be based on new and adequate information. The following are guides to style and form:

   (a) Manuscripts must be typed, double-spaced, and the ribbon copy submitted.

   (b) Pages must be numbered. The lines of text must be numbered in the left margin, beginning with one at the top of each page. This does not apply to tabular material.

   (c) Tables should be as few and as simple as is feasible for presentation of the essential data.

   (d) Legend for each figure should be typed on a separate sheet. Photographs should be carefully prepared and labeled. Magnifications of photomicrographs should be indicated on legends. Charts should be prepared in India ink. Typed material on charts is not acceptable. Material that will not reproduce satisfactorily will be returned for revision. For suggestions on preparing illustrative material see the "Style Brief" published by the Wistar Institute Press, Philadelphia. If in doubt, consult the Editor before putting material in finished form.

   (e) The introduction should consist only of a paragraph indicating very briefly the nature of the question asked and the reason for asking it. Reference to relevant literature should be made later in the discussion of the results. Concise presentation of data is desired and there should be evidence of statistical examination wherever obviously useful in the interpretation. Unsupported hypotheses should be avoided. The organization of a manuscript may vary with the nature of the material, but all manuscripts should contain a clear statement of application of the results or their relation to some problem of the livestock industry. Each paper should end with a brief summary in the form of an abstract containing the most pertinent data in the briefest possible form.

   (f) Literature citations must be listed in alphabetical order and should be referred to in the text by author and date, i.e., Castle (1924), or (Castle, 1924). Citations may be referred to by number in review articles where the number of citations is too great to permit convenient use of the above form. "Literature cited" lists should be typed in a style used in a current issue of the JOURNAL.

4. Manuscripts will be edited in the order received, and accepted papers will be published as nearly in this order as feasible.

5. There will be a charge for making engravings in excess of one page per paper and of printing tables in excess of two pages per paper. The author will be asked when his manuscript is accepted to indicate the institution, agency or individual that will assume responsibility for the charges and for the reprints ordered at that time.

6. Authors will receive galley proof of articles together with a form for supplying an abstract for "Biological Abstracts". *Corrected proof and abstract should be mailed to the Editor within three days after they are received. Failure to do this may result in delay of publication until the next issue.*

7. One author of each manuscript should be a member of the Society. Papers by non-members can be published only after approval by the Executive Committee.

8. A "News and Notes" section will be published in each issue, including Society announcements, changes in positions and other items of professional interest concerning the members, and other items of interest to the membership. All such items should be sent promptly to the Editor.

9. Abstracts of papers to be presented at the annual meetings of the Society will be published in the November issue. Such abstracts must not exceed 250 words in length, should be typed (double spaced) and the ribbon copy submitted, the lines on the page should be numbered, in the left margin, from top to bottom, and must be in the hands of the Secretary of the Society by August 15, preceding the meeting. These abstracts must be summaries of results and conclusions.

10. Presentation of a paper at the annual meeting does not exclude it from publication in complete form in the JOURNAL. Such papers may be submitted to the Editor and will be given equal consideration with others submitted.

11. All manuscripts and correspondence concerning them should be addressed to the Editor: Dr. L. E. Casida, Department of Genetics, University of Wisconsin, Madison, Wisconsin, or to the publishers: Boyd Printing Company, 372–374 Broadway, Albany 7, New York.

12. All correspondence concerning subscriptions to the JOURNAL OF ANIMAL SCIENCE and other business matters should be addressed to the Business Manager, Dr. J. I. Miller, Department of Animal Husbandry, Cornell University, Ithaca, N. Y., or to the publishers, Boyd Printing Company, 372–374 Broadway, Albany 7, New York.